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RESEARCH ARTICLE

Fertilizing the Flame: Effects of AB Fertilizer Concentration on Vegetative Growth, Fruit Yield, and Capsaicin Biosynthesis in Capsicum frutescens

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Abstract The *Capsicum* crops, known for their diverse phytochemical composition and pungency, are significantly influenced by agricultural practices. This study presents research on the 'cili padi' variety of *Capsicum frutescens,* a relatively unexplored species, grown hydroponically on a cocopeat medium using a high-quality AB fertilizer with varied nutrient concentrations. The research focused on analysing plant growth patterns, fruit yield, and capsaicinoid levels throughout the growth cycle. This involved detailed morphological measurements, productivity assessments, and the use of cyclic voltammetry for capsaicinoid quantification. The findings revealed that vegetation growth, yield, and metabolite parameters were closely linked to fertilizer concentration. Notably, an increase in nutrient availability led to improvements in plant growth metrics, fruit yield, and capsaicin levels. However, these benefits peaked within a specific fertilizer concentration range (1.5-2.1 E.C.-mS/cm) before declining, indicating the existence of toxicity thresholds. The study highlights the critical importance of balanced fertilization in hydroponic cultivation of C. frutescens, as it simultaneously enhances productivity and pungency quality. Interestingly, the research uncovered a threshold in nutrient concentration that optimizes nitrate conversion into capsaicin, beyond which overall plant growth continues to increase with higher fertilizer quantities. This finding necessitates further investigation into the metabolic and transport pathways that limit phytochemical production in these plants. Additionally, the capsaicinoid concentrations achieved in this study resulted in only moderately pungent Scoville ratings, suggesting a variance in biosynthetic capacity between this specific local species and more commonly studied pepper types. In summary, this research underscores the importance of a well-calibrated fertilization strategy to effectively boost both yield and pungency quality in *C.frutescens* . The insights gained from this study contribute significantly to our understanding of optimal hydroponic cultivation practices for enhancing the desirable traits of this unique chili variety.

Keywords: *Capsicum frutescens*, AB fertilizer, plant morphology, capsaicin, cyclic voltammetry, capsaicinoid.

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Introduction

The applications of chemical fertilisers significantly influences the chemical composition and nutritional quality of vegetables and plants. Excessive use of nitrogen-based fertilisers is known to increase nitrate and nitrite concentrations in vegetables, posing risks to their safety, nutritional value, and shelf life (Babalar 2023, Cintya 2018). This overuse can lead to an accumulation of nitrates in crops, negatively affecting the quality and longevity of fresh and minimally processed vegetables. Conversely, organic fertilisers have been shown to enhance the vitamin C content of vegetables, offering a nutritional advantage over their chemically fertilised counterparts. The relationship between nitrogen nutrition and vitamin C levels varies across plant species, with organic crops generally exhibiting higher vitamin C content than conventionally grown ones (Babalar 2023).

Chemical fertilisers also contribute to environmental concerns such as soil acidification, air and groundwater contamination, and damage to plant root systems (Saleem, 2023). Long-term reliance on these fertilisers can lead to soil degradation, nitrogen leaching, soil compaction, reduced organic matter in the soil, and carbon depletion (Lin 2019). In contrast, organic fertilisers are known to improve soil health and productivity, promoting sustainable agricultural practices.

The impact of chemical fertilisers extends to specific molecules in plants. For instance, varying nitrogen levels in fertilisers can alter the concentration of gingerols and shogaols in ginger, with higher nitrogen levels increasing the 6-gingerol content (Suhaimi 2021). The use of NPK fertilisers has been observed to affect the mineral nutrient composition in ginger, particularly increasing potassium, phosphorus, and sodium levels (Jabborova 2021). In garlic, sulphur and nitrogen-based fertilisers influence the chemical composition of allicin, with increased sulphur leading to higher alliin concentrations in both leaves and bulbs (Bloem 2005; Nguyen 2022). Similarly, sulphur fertilisation enhances allicin presence in garlic, with higher sulphur rates correlating with increased allicin content (Thangasamy 2021, Nguyen 2021). The effect of chemical fertilisers on piperine in black pepper has also been studied, with research indicating that a combination of bio-chemical fertilisers can improve the agricultural and biochemical properties of black cumin (*Nigella sativa* L.) (Moradzadeh 2021).

Chilli, a widely cultivated plant, is renowned for its pungency, primarily due to capsaicin (Reyes-Escogido *et al* 2011). The pungency level, measured in Scoville Heat Units (SHU), varies across species and is influenced by environmental factors, soil type, and ripeness. The global trend towards spicy foods, exemplified by products like “McDonald 3x Spicy” and “Maggi Pedas Giler,” has increased the commercial demand for hotter chilies (Spence, 2018). The hotter the chilli, the higher the SHU. This trend, coupled with a rise in hot and spicy product launches, presents new opportunities for agricultural research and production.

Currently, most studies on chilli have concentrated on the *Capsicum annuum* variety. *C.annuum* is the most widely studied *Capsicum* species compared to other *Capsicum* varieties due to its commercial importance as a widely cultivated crop and its high variability in terms of fruit shape, size, colour, and pungency levels. (Hernández-Pérez, 2020). In this study, *C.frutescens* , or bird’s eye chilli. *C.frutescens* possesses several unique characteristics and qualities: It is known for its high pungency, contributing to its spiciness. It is often described as having a distinctive flavour that adds a deeper taste to local foods in tropical regions (Yamamoto 2004, Rahim *et al.* 2012).). The plant typically grows to a height of 10–20 millimetres and produces small, pungent berries that are usually erect and grow to a length of 10–20 millimetres. The fruit starts as a pale yellow and matures to a bright red, although other colours are also possible. The species has a smaller variety of shapes compared to other *Capsicum* species. *C.frutescens* is a wild chilli pepper with genetic proximity to the cultivated pepper *Capsicum chinense*, and it is native to Central and South America, with the Tabasco variety being one of the most well-known. This subspecies is often used for making hot sauces due to its pungency and flavour. It is also suitable for cultivation in pots and in cooler climates.

The separation of nutrients in hydroponic fertilisers like the AB system has evolved from early experimentation and scientific understanding of plant nutrition. In the early 1900s, pioneering researchers like William Gericke were developing the foundations of hydroponics. They initially used single-solution fertilisers, often based on modified soil formulas. However, problems arose due to chemical incompatibilities. Certain nutrient combinations, like calcium and phosphate, would react and form insoluble precipitates, making them unavailable to plants. This led to nutrient deficiencies and hindered plant growth. To address these issues, scientists like D.R. Hoagland and L.C. Chandler conducted extensive research in the 1930s and 1940s (Biale 1978). They identified specific nutrient groups that reacted negatively and studied their solubility characteristics. Based on their findings, they proposed the concept of "balanced solutions." These solutions separated incompatible nutrients into two distinct parts, preventing precipitation and ensuring availability for plant uptake. The A and B nutrient systems emerged as a practical application of these scientific principles. By separating key nutrients (macronutrients in A and micronutrients and phosphates in B), growers could easily mix them in precise ratios just before use, preventing unwanted reactions. The benefits were clear: improved nutrient uptake, optimised plant growth, and greater control over nutrient balance. This led to the widespread adoption of A and B systems by hydroponic hobbyists and commercial growers alike.

This study aims to explore the effects of different AB chemical fertiliser dosages on the growth and capsaicin production of *C.frutescens*. The study focused on various growth parameters, including plant height, chlorophyll content, leaf count, and size, and quantified the yield in terms of fruit quantity, size, and mass. Capsaicin levels were measured using cyclic voltammetry, and the pH of the cocopeat growth medium was monitored to assess the overall impact of chemical fertilisers on chilli plant development and capsaicin synthesis.

Experimental

Plant Material and Growth Medium

For this study, 60 *C.frutescens* plants were sourced from a reputable local vendor NZ Kota in Ayer Hitem, Johor. Cocopeat, chosen for its favourable properties as a growth medium, was used to support plant growth (Awang *et al* 2009). The plants were three weeks old when it was carefully transplanted into the cocopeat during the cooler hours of early morning and evening to minimise stress from direct sunlight.

Fertigation System

A sophisticated drip irrigation system was designed to deliver varying concentrations of AB fertiliser to the plants. This system comprised an automatic irrigation controller, a water pump, a filter, valves, high-density polyethylene (HDPE) hoses, and arrow drippers, ensuring precise and consistent watering (Fan 2017). The system was calibrated to provide a 2-liter per hour drip feed to each plant (Phocaides 2007, Samsuri 2010). The AB fertiliser used in this study was a commercial blend selected for its compatibility with *C.frutescens*.

The Fertiliser Concentration and Application

The concentration of the AB fertilizer solutions was modulated over the course of the *C.frutescens* growth phases, with overall nutrient strength and dosing frequency increased aligned with plant maturity. The electrical conductivity (EC) in mS/cm units was used as a proxy for monitoring fertilizer salt ion levels and strength. EC values were escalated on a timed program ranging from 1.2 up to 2.5 mS/cm from early vegetative establishment through fruit ripening respectively. A control set with 0 EC at zero fertilizer concentration was maintained for comparative evaluation. Three test concentrations tracks were set, with the moderate C2 EC regimen maximizing at 1.8 mS/cm by the flowering stage. Careful elevation of fertilizer dosage via EC tracking aimed to balance providing adequate nutrition for developmental needs against toxicity threats from excess.

Table 1 Composition of commercial AB fertilizer

A	B
Calcium nitrate Iron chelates	Potassium nitrate Monopotassium phosphate Magnesium sulphate Manganese (II) sulphate Copper (II) sulphate Zinc sulphate Sodium molybdate

Table 2 Concentration and frequency of AB fertilizer using fertigation system

System	EC value (E.C.-mS/cm)							
	W1	W2	W3	W4	W5	W6	W7	W8
Control	0	0	0	0	0	0	0	0
C1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
C2	1.2	1.2	1.2	1.3	1.3	1.5	1.8	2.0
C3	1.2	1.2	1.5	1.7	1.9	2.1	2.3	2.5

Morphological Observations and pH Measurement

Weekly observations were conducted to monitor plant morphology. Plant height was measured in centimetres using a standard ruler. Chlorophyll content was quantified using a chlorophyll metre. The leaf count and dimensions were recorded, measuring from the leaf tip to the point of attachment on the stem. The pH of the cocopeat was regularly monitored using a pH metre, ensuring optimal growing conditions.

Capsaicin Extraction

Capsaicin extraction followed the method outlined by Ananthan, Subash, & Longvah (2014). Freshly harvested chilli samples, weighing 12 grammes, were finely chopped and macerated in 30 ml of methanol. This mixture was then heated at 70 °C for 5 hours in a water bath. Post-extraction, the volume was adjusted to 25 mL with methanol. The mixture was centrifuged at 4000 rpm for 10 minutes at 1 °C, and the supernatant was filtered through a 0.22 µm syringe nylon filter for purity.

Capsaicin Detection

Capsaicin levels were detected using cyclic voltammetry, as described by Supalkova et al. (2007). The setup included a glass-carbon electrode as the working electrode, a platinum wire counter electrode, and a silver wire reference electrode. The supporting electrolyte was an acetate buffer with a pH of 4.0, prepared by mixing 0.1 mol of sodium acetate with 0.1 mol of acetic acid. Sodium hydroxide and hydrochloric acid were used to adjust the pH as needed. For the analysis, 5 mL of the sample was introduced into 50 mL of the supporting buffer. The voltammetry parameters were set as follows: upper peak at 0 V, lower peak at 0.9 V, step potential at 5 mV, and scan rate at 0.1 V/s, with all experiments conducted at a controlled temperature of 25 °C.

Statistical Analysis

The statistical difference between the treatments was determined using a one-way analysis (ANOVA) with a factor at a significance level of ($p< 0.05$). In the one-way ANOVA, the null hypothesis is rejected if the P value is less than 0.05 ($p < 0.05$) and the f value is greater than the critical value f. This means that the data are significantly different. The null hypothesis must always contain some form of equality. In this study, the null hypothesis is that the mean value between all concentrations (C1, C2, and C3) is the same. The ANOVA test is performed to determine the effects of different concentrations of chemical fertilisers on plant growth.

Results and Discussion

Impact of Chemical Fertiliser Concentrations on Morphological Characteristics of *C.frutescens*

Plant Height Dynamics

Throughout the study, a progressive increase in the average plant height for the three fertiliser concentration groups (C1, C2, and C3) was observed. Notably, the C3 group, which received the highest fertiliser concentration during the planting phase, initially exhibited the most rapid growth. This rapid growth can be attributed to the high availability of essential nutrients, particularly nitrogen, which is crucial for plant development (Daniel-Vedele, Krapp, & Kaiser, 2010). However, in week 4, a slight decline in the average height of the C3 group was observed, likely due to the increased susceptibility of these plants to diseases, a side effect of the high fertiliser concentration.

Statistical analysis using ANOVA confirmed the significant impact of fertiliser concentrations on plant height, with a p-value less than 0.05 ($p < 0.05$) and an f-value exceeding the critical threshold. This underscores the influence of NPK components—nitrogen, phosphorus, and potassium—on plant growth, aligning with findings by Bandyopadhyay et al. (2010) and Moneruzzaman Khandaker et al. (2017), who highlighted the essential role of these nutrients in early plant development stages.

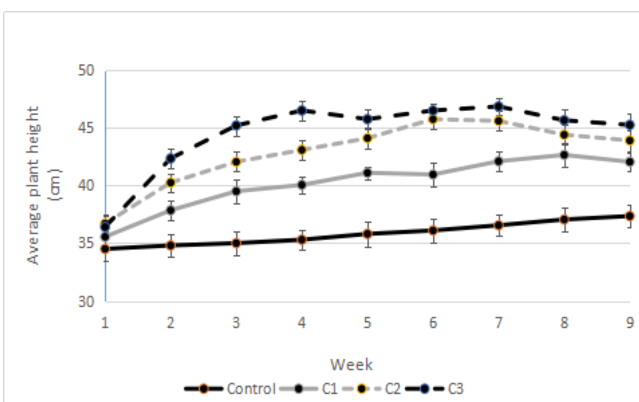

Figure 1. Effect of different concentration of chemical fertilizer on plant height

Leaf Count Variations

Similar to plant height, the average number of leaves for groups C1, C2, and C3 showed an increasing trend over the weeks. However, a notable decrease in leaf count was observed in the C3 group during week 6. This decline can be linked to the high concentration of fertilizer used in this group, which, while promoting rapid initial growth, also increased the plants' vulnerability to diseases. The role of nitrogen in fertilizer in chlorophyll formation and subsequently in leaf production is well-documented (Moneruzzaman Khandaker et al., 2017), yet the adverse effects of excessive fertilization cannot be overlooked. The results of the ANOVA analysis further reinforced these observations, indicating a statistically significant effect of fertilizer concentrations on the number of leaves, as evidenced by a p-value less than 0.05 ($p < 0.05$) and an f-value above the critical value. This finding is consistent with the established understanding of the critical role of NPK in plant growth and leaf development.

The results showed that higher chemical fertiliser concentrations enhanced vegetative growth of *C.frutescens*, as indicated by increased plant height and leaf count over time, but also apparently escalated disease vulnerability, as evident from sudden decline starting Week 6 for C3. This alignment between nutrient availability and growth metrics is consistent with the literature demonstrating the pivotal role of nitrogen, phosphorus, and potassium in facilitating plant development. The data suggests an optimum balance around concentration C2, which stimulated robust height and leaf production without subsequent rapid drops. Further studies across a spectrum of fertility levels can better define growth optimisation points by balancing nutritional needs with immunity.

The observed decreases in weeks 4 and 6 for height and leaf averages, respectively, imply increased pathogenesis at higher fertiliser doses. Nutrient excesses or imbalances may have disrupted physiological processes or reduced defensive capacity. Since leaf counts were impacted slightly later than plant height, leaves may have a higher priority for nutrients and thus be temporarily protected compared to overall plant stature. Alternatively, height may involve greater metabolic demands and thus be more sensitive to dysfunction from disease onset caused by excessive fertilizer conditions.

The main components of chemical fertilisers are NPK, an acronym for nitrogen, phosphorus, and potassium (Bandyopadhyay, Misra, Ghosh & Hati, 2010). Every one of these essential elements serves a vital role in plant nutrition. Nitrogen is the predominant nutrient necessary for plant development, since plants assimilate more nitrogen than any other element (Daniel-Vedele, Krapp & Kaiser, 2010). The inclusion of nitrogen, phosphorus, and potassium in fertilisers has a substantial impact on the growth and maturation of plants (Moneruzzaman Khandaker, Rohani, Dalorima & Mat, 2017). The NPK, also known as essential nutrients, play a vital role in fostering robust plant development throughout the first phases. Inadequate concentrations of these nutrients can impede plant growth and progress (Moneruzzaman Khandaker, Rohani, Dalorima & Mat, 2017). The presence of essential elements such as nitrogen, phosphorus, and potassium have an impact on the growth of *C.frutescens* plants. Nitrogen is essential for controlling the levels of chlorophyll and the colour of immature fruit in pepper plants (Brand et al., 2014). Additionally, the research highlights the impact of nitrogen on the quality of seedlings of *C.frutescens*, underscoring its importance during the initial phases of plant growth (Silva et al., 2021). Furthermore, the impact of potassium and calcium nitrate on the growth and development of *C.frutescens*, a crucial factor in producing superior pepper seedlings, has been investigated (Silva et al., 2021).

Nitrogen availability plays a crucial role in the production of secondary metabolites, including capsaicinoids, in *Capsicum* species. Several studies have investigated the influence of nitrogen fertilization on capsaicin content in various pepper cultivars. Gurung et al. (2011) reported that nitrogen application significantly affected capsaicin levels in *C. annuum*, with higher nitrogen rates resulting in increased capsaicin content up to a certain level. Similarly, Monforte-González et al. (2010) found that nitrogen fertilization enhanced capsaicinoid accumulation in habanero pepper (*C. chinense*). The authors suggested that nitrogen availability influences the activity of key enzymes involved in the capsaicin biosynthetic pathway, such as phenylalanine ammonia-lyase (PAL) and capsaicin synthase (CS). Ancona-Escalante et al. (2013) also demonstrated that nitrogen fertilization increased capsaicin content in *C. chinense*, potentially by upregulating the expression of capsaicin biosynthesis-related genes. These studies highlight the significant impact of nitrogen on secondary metabolite production in *Capsicum* species, supporting our findings on the relationship between AB fertilizer concentrations and capsaicin content in *C. frutescens*.

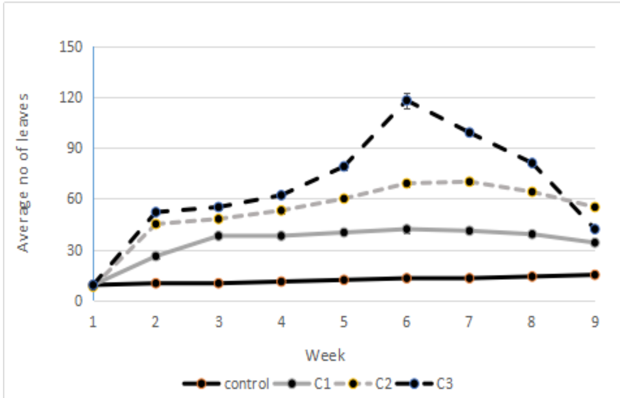

Figure 2. Effect of different concentration of chemical fertilizer on number of leaves

Fruit Harvest Quantity

Our study revealed a direct correlation between the concentration of fertiliser and the yield of *C.frutescens* . Group C3, which received the highest fertiliser concentration, yielded the largest average number of fruits, while Group C1, with the lowest fertiliser concentration, had the smallest yield. This trend aligns with the findings of Medina-Lara et al. (2008), who reported an increase in fruiting with higher nitrogen concentrations in fertilisers. The increase in the number of harvested fruits as the nitrogen content in the fertiliser rises further supports the critical role of nitrogen in fruit production. Statistical analysis using ANOVA confirmed the significant impact of fertiliser concentrations on the yield, with a p-value less than 0.05 ($p < 0.05$) and an f-value exceeding the critical threshold. This indicates that varying concentrations of chemical fertilisers have a statistically significant effect on the number of fruits harvested from *C.frutescens.*

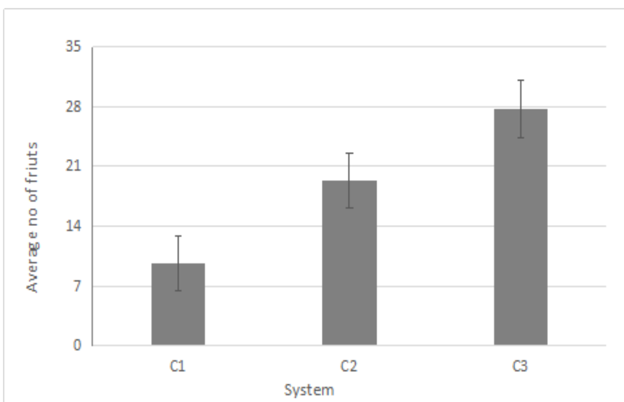

Figure 3. Effect of different concentration of chemical fertilizer on number of *C.frutescens* harvested

Fruit Harvest Quantity

The study also investigated the impact of different fertiliser concentrations on the mean weight of harvested *C.frutescens* fruits. The average mass of collected fruits followed a consistent pattern, with group C3 having the largest mass, followed by C2 and C1. This indicates that increased concentrations of fertiliser not only augment the quantity of fruits, but also improve their dimensions and weight. The results are consistent with the findings of Moneruzzaman Khandaker et al. (2017), who reported comparable outcomes in chilli plants subjected to organic fertiliser. The ANOVA findings confirmed these observations, showing a substantial impact of fertiliser concentrations on the mass of harvested fruits. This was demonstrated by a p-value less than 0.05 ($p < 0.05$) and an f-value exceeding the critical threshold. This study substantiates that varying doses of chemical fertilisers have a substantial impact on the yield of *C.frutescens* fruits.

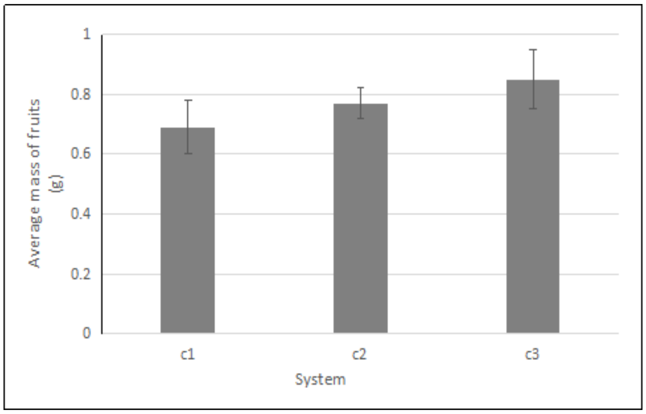

Figure 4. Effect of different concentration of chemical fertilizer on mass of *C.frutescens* harvested

The quantity and weight of harvested *C.frutescens* fruits exhibited an upward trend with increasing concentrations of chemical fertilisers, reaching their highest point between C2 and C3, without any further improvement beyond that. The added fertility likely improved the reproductive capability and growth by providing essential nitrogen, phosphorus, and potassium, which are necessary for flowering and fruit production. Nevertheless, the fact that the yields have reached a plateau despite the increased nutrient levels suggests that there may be a decrease in effectiveness beyond the ideal amounts, maybe caused by imbalances that produce hazardous reactions. Additional study that thoroughly examines crop yield at various levels of standard to extreme conditions might provide specific thresholds for optimisation.

In contrast to the steady advancements in height and leaf count, the quantity of fruit reached its highest point between C2 and C3 and then stayed constant. The observed variation indicates that resources are allocated preferentially to reproductive outputs in response to dietary stress. Quantitative molecular investigations that measure alterations in gene expression over a period of time can offer valuable insights into the internal division of a system.

The significant influence of AB fertilizer concentrations on plant height, leaf count, and fruit yield in *C.frutescens* is consistent with the findings of previous studies. Medina-Lara et al. (2008) reported that increasing nitrogen fertilization led to higher plant height, leaf area, and fruit yield in habanero pepper (*Capsicum chinense* Jacq.). Similarly, Ayodele et al. (2015) observed that increasing NPK fertilizer rates resulted in improved growth and yield parameters in C. annuum. The positive correlation between nitrogen availability and vegetative growth can be attributed to the essential role of nitrogen in chlorophyll synthesis, photosynthesis, and protein formation (Marschner, 2012). However, our study also revealed that excessive nitrogen fertilization (C3 treatment) led to a slight decline in growth parameters, possibly due to nutrient imbalances or increased susceptibility to diseases, as reported by Huber and Thompson (2007).

While revealing key influences on productivity, this initial study was somewhat limited in exploring only three fertiliser conditions lacking a lower control threshold. Subsequent experiments should broaden the scope of this concentration range in order to examine the chilli yield responses throughout a spectrum ranging from insufficient to abundant amounts. Examining this can enhance our comprehension of the threshold at which reproductive development transitions from augmentation to inhibition as fertility increases. The findings demonstrated that increasing the quantities of chemical fertilisers resulted in a higher yield and larger size of *Capsicum* fruits. However, the benefits reached a point of saturation, suggesting that there is an ideal range for productivity that should not be exceeded to avoid toxicity. By conducting extensive fertility trials and genetic profiling, a more precise understanding of how different concentrations affect the reproductive capacity of chilli crops and identify any trade-offs with plant health can be gained.

pH Dynamics of Growth Medium

The pH of the coco peat growth medium remained close to the normal 6.5-7.0 range optimal for *C.frutescens* crop cultivation under the C1 and C2 fertilizer concentrations over the 8-week duration. However, a drastic declining trend was elicited by the C3 regime from week 4 through week 8 (Figure 5). This acidification effect is attributable to excessive build-up of nitrates whose inherent acidic property manifests under high accumulation, overwhelming natural buffering capacity. Statistical testing via one-way ANOVA verified significant impacts of the fertilization treatments on medium pH, as seen from obtained p-values below the 0.05 threshold and F-statistics exceeding critical values. Together, the data indicates a need to strategize nutrient dosing, particularly nitrogen contribution, to preclude unfavourable acidic shifts and maintain media conditions conducive for pepper growth. Further expanding concentration gradients can delineate pH stability thresholds more precisely.

From Figure 5, there is a clear effect of the different chemical fertiliser concentrations (C1, C2, and C3) on the pH of the coco peat growth medium over the 8-week duration of the experiment. In essence, the controlled pH suggests C2 nutrient levels and accumulating rates still fall within cellular and metabolic tolerance limits for *Capsicum* plants to selectively draw favourable ions and exude acidity regulation byproducts. The more mature and expansive root system of *C.frutescens* plants at week 7 compared to earlier weeks would enable enhanced nutrient uptake capacity without adverse effects. As roots grow over time, the plants can tap into greater soil volumes, accessing more available fertiliser reserves. The larger surface area allows for higher uptake rates to meet the metabolic demands of larger plants. Extensive roots also likely secrete more acid regulators, modulating pH changes. Transport efficiency to foliage may also improve via well-formed vascular bundles, enabling utilisation rather than accumulation. Essentially, the root growth enables sufficient fertiliser acquisition without excessive buildup. This is coupled with proportionate biomass generation utilising the absorbed nutrients. This developmental stage-specific enhancement in nutrient access and utilisation to prevent toxicity adds an important temporal dimension to interpreting concentration thresholds. Monitoring root morphology and quantifying mineral distributions would provide further insights. In contrast, under the highest concentration of C3, the pH dropped sharply from week 4 through week 8 (Figure 5). Excessive nutrient accumulation due to the inability to absorb it, like in C2, especially nitrates, likely caused increasing acidification due to the inherently acidic properties of nitrogen compounds at higher levels. A one-way ANOVA confirmed the significant effects of the fertiliser regimes on medium pH.

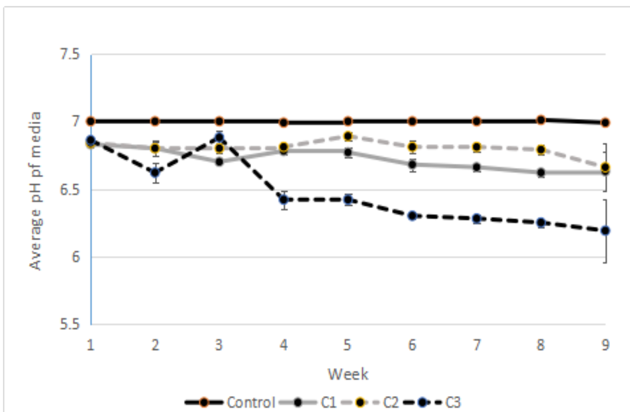

Figure 5. Effect of different concentration of chemical fertilizer on pH of media

In summary, the highest fertiliser concentration of C3 resulted in excessive acidification of the media, which took it outside the preferred pH range for chilli crops. This can negatively impact nutrient availability and induce physiological dysfunction. Follow-up studies should track pH across a greater concentration breadth, along with titrating acids and bases, to pinpoint optimal pH domains for balancing both chilli growth and necrotic losses from extremes. Maintaining media pH within favourable bands through fertilisation adjustment can prevent nutrient imbalances and toxicity, given that most crops have distinct pH preferences.

Capsaicin Quantification

Capsaicin levels quantified via cyclic voltammetry culminated at concentration C2, declining slightly thereafter (Figure 6, Table 3). These parallels optimized vegetative development and fruit production at C2 compared to C3. As an essential input regulating secondary metabolism, nitrogen availability drives pungent capsaicin accumulation up to a point before likely inducing toxicity from excess. Past studies confirm nitrogen impacts on capsaicin biosynthesis in peppers (Medina-Lara et al., 2008). The reduction from C2 to C3 indicates possible inhibitory effects on phytochemical synthesis beyond optimal fertilization.

Using the calibration curve in Figure 7, cyclic voltammetry was used to measure capsaicin levels. The highest levels were found in C2 regime fruits. Peak current correlates with capsaicin content (Randviir et al., 2013). Studies have demonstrated that nitrates stimulate the production of capsaicinoids (CAPs). The strong flavour or pungency of chilli peppers pods is attributed to the chemicals synthesised from phenylalanine and branched amino acids, which are produced by the placental tissue (Aldana-luit 2015). In line with previous research, higher amounts of nitrate in fertiliser increased capsaicin biosynthesis at first, but higher amounts did not lead to higher accumulation (Medina-Lara et al., 2008), which probably means there are toxicity thresholds. The capsaicin content in the sample was converted into Scoville heat units (SHU) using the following equation (Baytak & Aslanoglu, 2017).

SHU = concentration of capsaicin (ppm) x 16

The pungency of *C.frutescens* in C2 is the highest at 2414.88 SHU, qualifying as mildly spicy on the Scoville scale between 700 and 3000 SHU, while it is the lowest in C1 at 1735.52 SHU, and C3 is in the middle at 2035.68 SHU. There are five levels of spiciness classified by Scoville heat units (SHU): not spicy (0–700 SHU), slightly spicy (700–3,000 SHU), moderately spicy (3,000–25,000 SHU), very spicy (25,000–70,000 SHU), and very spicy (> 80,000 SHU) (Othman, Ahmed, Habila, & Ghafar, 2011, Naves 2019). Although C3 had lower heat than C2, both surpassed C1 levels, affirming nitrate form and dosage as regulators of capsaicinoid accumulation. However, the actual SHU ratings were still low compared to the most extreme *Capsicum* phenotypes. This shows how important it is to balance cultivation interventions with the plant's natural bio-synthetic limits.

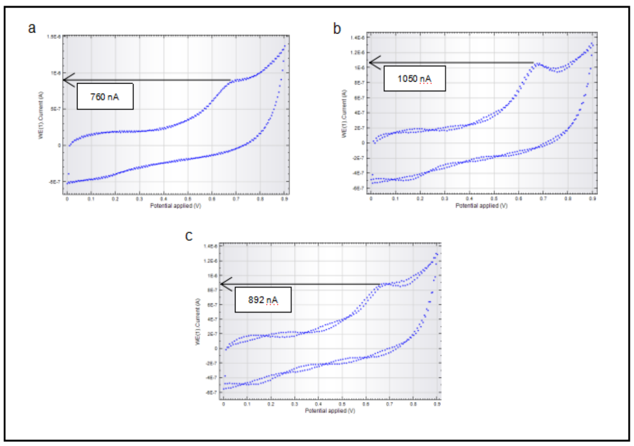

Figure 6. Voltammograms for detection in capsaicin at approximately 0.67V. a) C1, b) C2, C) C3

The observed variations in capsaicin concentrations in *C.frutescens* , as determined by cyclic voltammetry, offer intriguing insights into the impact of fertilization on phytochemical synthesis. Notably, capsaicin levels peaked at the C2 fertilizer concentration and then marginally declined (Figure 6 and Table 3), mirroring the optimized vegetative growth and fruit production observed at this concentration compared to C3. This trend suggests a nuanced relationship between nitrogen availability and secondary metabolism, where nitrogen enhances capsaicin synthesis up to a threshold, beyond which it may become detrimental, possibly due to toxicity from excessive accumulation. This phenomenon aligns with previous studies highlighting nitrogen's influence on capsaicin biosynthesis in peppers (Mediana-Lara et al., 2008). The decline in capsaicin levels from C2 to C3 hints at potential inhibitory effects on phytochemical synthesis when fertilizer application exceeds the optimal level.

The variation in capsaicin content among the different nitrogen treatments can be attributed to the role of nitrogen in the biosynthesis of this secondary metabolite. Nitrogen is essential for the synthesis of amino acids, which serve as precursors for capsaicinoid production. The capsaicin biosynthetic pathway involves the convergence of two distinct pathways: the phenylpropanoid pathway, which provides the vanillylamine moiety, and the branched-chain fatty acid pathway, which contributes the acyl chain (Mazourek et al., 2009; Gurung et al., 2011). Phenylalanine, an aromatic amino acid, is the starting point for the phenylpropanoid pathway. Adequate nitrogen availability ensures a sufficient supply of phenylalanine for capsaicin synthesis. Moreover, nitrogen is involved in the formation of key enzymes, such as phenylalanine ammonia-lyase (PAL) and capsaicin synthase (CS), which catalyze crucial steps in the capsaicin biosynthetic pathway (Gurung et al., 2011; Zhang et al., 2016). Therefore, the variation in nitrogen levels across the treatments likely influenced the activity of these enzymes and the overall capsaicin production in *C.frutescens.*

Table 3 Peak current corresponding to the concentration of the capsaicin in every sample

System	Potential Applied (V)	Peak Current (nA)	Concentration of capsaicin (ug/mL)
C1	0.67	760	108.47
C2	0.67	1050	150.93
C3	0.67	892	127.23

Another critical aspect is the developmental stage at which the chilli fruits were harvested. The initial harvest, conducted 6 weeks post-transplantation, might not have coincided with the peak of capsaicin biosynthesis, typically occurring in later maturity stages. Harvesting at this relatively early stage could have precluded capturing the maximum heat levels, as capsaicinoid synthesis intensifies during the full maturity transition, particularly during the green to intermediate orange stages marked by carotenoid accumulation and sharp spikes in capsaicinoid levels.

The study also observed a continuous improvement in certain parameters (fruit count, leaf number) while capsaicin levels plateaued between the C2 and C3 fertilizer doses. This could be attributed to distinct stress responses in the plants. While C3 plants were more productive, they accumulated fewer capsaicinoids than C2 plants, possibly due to excessive nutrients diminishing the levels of signalling molecules that stimulate capsaicin defence in response to reduced biotic/abiotic stressors. Moreover, surpassing the optimal nitrogen level could lead to toxicity effects that specifically inhibit capsaicin production pathways. Additionally, an elevated nutritional status might alter resource allocation, favouring growth over secondary metabolism. The cili padi variant's genetic or developmental constraints in redirecting excess precursors from an overflow of energy towards capsaicinoids could also play a role.

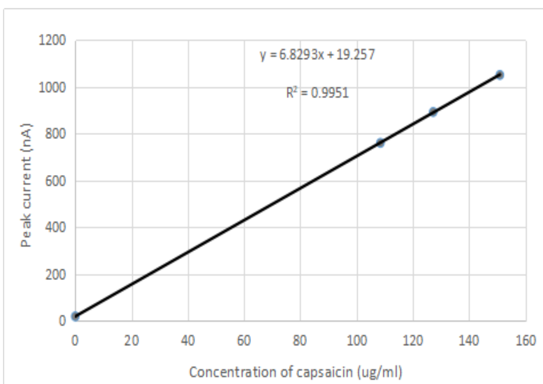

Figure 7. Calibration curve for the determination of the relationship between peak current (nA) and concentration of capsaicin (ug/ml).

The study's findings underscore the influence of various growth environments on chili pungency, determined by capsaicin content. Factors such as drought stress, altitude, rainfall, temperature, cultivar selection, and soil properties significantly impact capsaicin concentration in chilies (Gurung 2011, Mahmood 2021). In this research, the absence of environmental stressors other than fertilizer application suggest that regional chili varieties may not reach the extreme heat levels of some other *Capsicum* cultivars such as the local Semerah Padi variety, that has a moderate SHU of ~6200 (Suhana 2019). This could be due to inherent genetic limitations in the biosynthetic capacity of this particular *Capsicum* accessions, restricting its potential for high pungency despite growth factor modulation like fertilisation. Furthermore, the choice of cocopeat as the planting medium could have influenced the results. Cocopeat, increasingly used as an eco-friendly alternative to peat in soilless growth media, typically has low levels of micronutrients and mineral nitrogen, such as calcium and magnesium (Meerow, 1995; Evans et al., 1996; Abad et al., 2002). Its high-water retention capacity, potentially leading to suboptimal air-water balance and inadequate aeration (Mathowa 2017), may have contributed to the lower total SHU values observed in the C1, C2, and C3 plants grown on cocopeat.

Analysis of capsaicin complements growth and yield results in revealing an apparent concentration ceiling for maximizing plant productivity and phytochemical quality. This highlights the importance of holistic crop research measuring multiple morphological and biochemical traits. As capsaicin confers protective and ecological roles for the chili plant, in addition to commercial pungency value, simultaneously optimizing its levels alongside yields is imperative.

The findings of this study underscore the importance of optimizing nitrogen fertilization for both plant growth and capsaicin content in *C.frutescens* cultivation. The results demonstrate that nitrogen levels significantly influence vegetative growth, fruit yield, and capsaicin concentration. However, it is crucial to strike a balance between promoting growth and achieving the desired pungency. Excessive nitrogen fertilization, as observed in the C3 treatment, led to a decline in capsaicin content, possibly due to metabolic imbalances or a shift in resource allocation towards vegetative growth rather than secondary metabolite production. On the other hand, insufficient nitrogen, as seen in the C1 treatment, resulted in suboptimal growth and lower capsaicin levels. Therefore, the C2 treatment, which provided a moderate nitrogen concentration, emerged as the optimal level for simultaneously enhancing growth and capsaicin content in *C.frutescens.* This study contributes to a better understanding of the nitrogen requirements for cultivating this specific chili variety, enabling farmers and researchers to fine-tune fertilization strategies for achieving the desired balance between yield and pungency.

Conclusion

This study demonstrated significant influences of chemical fertiliser concentration on growth patterns, fruit yield, and capsaicin phytochemical levels in *C.frutescens* chilli plants. Elevating nutrient availability drove improvements in vegetation metrics, quantities of harvested fruits, and capsaicin concentrations up to an apparent optimum at C2 fertility levels before declining or plateauing. The data showcases the importance of balanced fertilisation in simultaneously enhancing productivity and pungency quality. However, limitations like a narrow concentration range (1.5-2.1 E.C.-mS/cm) and time of application warrant expanded trials defining crossover points from augmentation to attenuation for different crop parameters. Overall, the intricate interconnections between nutrient inputs, chilli growth phases, and metabolites underscore the need for holistic agronomic perspectives integrating *C.frutescens* crop yield, phytochemical content, and safety and tolerance thresholds. This pioneering work on this variety established key concentration-response patterns while highlighting knowledge gaps around refining optimal regimes and addressing these areas can greatly advance precision chilli cultivation to satisfy rising consumer demand for high-quality spicy produce.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] Aldana-Iuit, J. G., Sauri-Duch, E., Miranda-Ham, M. D. L., Castro-Concha, L. A., Cuevas-Glory, L. F., & Vázquez-Flota, F. A. (2015). Nitrate promotes capsaicin accumulation in *Capsicum* chinense immobilized placentas. *Biomed Research International,* 2015.

[2] Ananthan, R., Subash, K., & Longvah, T. (2014). Assessment of nutrient composition and capsaicinoid content of some red chilies. *Int. Food Nutr. Technol., 72*, 1-4.

[3] Ancona-Escalante, W. R., Baas-Espinola, F. M., Castro-Concha, L. A., Vázquez-Flota, F. A., Zamudio-Maya, M., & Miranda-Ham, M. L. (2013). Induction of capsaicinoid accumulation in placental tissues of Capsicum chinense Jacq. requires primary ammonia assimilation. *Plant Cell, Tissue and Organ Culture (PCTOC), 113*(3), 565-570.

[4] Ayodele, O. J., Alabi, E. O., & Aluko, M. (2015). Nitrogen fertilizer effects on growth, yield and chemical composition of hot pepper (Rodo). *International Journal of Agriculture and Crop Sciences, 8*(5), 666-673.

[5] Awang, Y., Shaharom, A. S., Mohamad, R. B., & Selamat, A. (2009). Chemical and physical characteristics of cocopeat-based media mixtures and their effects on the growth and development of *Celosia cristata*.

American Journal of Agricultural and Biological Sciences, 4(1), 63-71.

[6] Babalar, M., Daneshvar, H., Díaz-Pérez, J. C., Nambeesan, S., Tabrizi, L., & Delshad, M. (2023). Effects of organic and chemical nitrogen fertilisation and postharvest treatments on the visual and nutritional quality of fresh-cut celery (Apium graveolens L.) during storage. *Food Science & Nutrition*, *11*(1), 320-333.

[7] Brand, A., Borovsky, Y., Hill, T., Rahman, K. A. A., Bellalou, A., Van Deynze, A., & Paran, I. (2014). CaGLK2 regulates natural variation of chlorophyll content and fruit color in pepper fruit. *Theoretical and Applied Genetics, 127*, 2139-2148.

[8] Bandyopadhyay, K. K., Misra, A. K., Ghosh, P. K., & Hati, K. M. (2010). Effect of integrated use of farmyard manure and chemical fertilizers on soil physical properties and productivity of soybean. *Soil and Tillage Research*, *110*(1), 115-125.

[9] Baytak, A., & Aslanoglu, M. (2017). Sensitive determination of capsaicin in pepper samples using a voltammetric platform based on carbon nanotubes and ruthenium nanoparticles. *Food Chemistry, 228*(2017), 152-157.

[10] Biale, J. B. (1978). On the interface of horticulture and plant physiology. *Annual Review of Plant Physiology, 29*(1), 1-24.

[11] Bloem, E., Haneklaus, S., & Schnug, E. (2005). Influence of nitrogen and sulfur fertilization on the alliin content of onions and garlic. *Journal of Plant Nutrition*, *27*(10), 1827-1839.

[12] Cintya, H., Silalahi, J., Putra, E. D. L., & Siburian, R. (2018). The influence of fertilizer on nitrate, nitrite and vitamin C contents in vegetables.

[13] Daniel-Vedele, F., Krapp, A., & Kaiser, W. (2010). Cellular biology of nitrogen metabolism and signaling. *Plant Cell Monographs*, 145-172. Doi: 10.1007/978-3-642-10613-2_7.

[14] Fan, J., Wu, L., Zhang, F., Yan, S., & Xiang, Y. (2017). Evaluation of drip fertigation uniformity affected by injector type, pressure difference and lateral layout. *Irrigation and Drainage, 66*(4), 520-529.

[15] Gurung, T., Techawongstien, S., Suriharn, B., & Techawongstien, S. (2011). Impact of environments on the accumulation of Capsaicinoids in *Capsicum* spp. *HortScience*, *46*(12), 1576-1581.

[16] Hernández-Pérez, T., Gómez-García, M. D. R., Valverde, M. E., & Paredes-López, O. (2020). *Capsicum* annuum (hot pepper): An ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. A review. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 2972-2993.

[17] Huber, D. M., & Thompson, I. A. (2007). Nitrogen and plant disease. In: Datnoff, L. E., Elmer, W. H., & Huber, D. M. (eds). Mineral nutrition and plant disease. The American Phytopathological Society, St. Paul, MN, 31-44.

[18] Jabborova, D., Sayyed, R. Z., Azimov, A., Jabbarov, Z., Matchanov, A., Enakiev, Y., ... & Datta, R. (2021). Impact of mineral fertilisers on mineral nutrients in the ginger rhizome and on soil enzymes activities and soil properties. *Saudi Journal of Biological Sciences, 28*(9), 5268-5274.

[19] Lin, W., Lin, M., Zhou, H., Wu, H., Li, Z., & Lin, W. (2019). The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards. *Plos One, 14*(5), e0217018.

[20] Mahmood, T., Rana, R. M., Ahmar, S., Saeed, S., Gulzar, A., Khan, M. A., ... & Du, X. (2021). Effect of drought stress on capsaicin and antioxidant contents in pepper genotypes at reproductive stage. *Plants, 10*(7), 1286.

[21] Marschner, H. (2012). *Marschner's mineral nutrition of higher plants* (3rd ed.). Academic Press, London, UK.

[22] Mathowa, T., Tshipinare, K., Mojeremane, W., Legwaila, G. M., & Oagile, O. (2017). Effect of growing media on growth and development of sweet paper (*Capsicum* annum L.) seedlings. *Journal of Applied Horticulture*, *19*(3), 200-204.

[23] Mazourek, M., Pujar, A., Borovsky, Y., Paran, I., Mueller, L., & Jahn, M. M. (2009). A dynamic interface for capsaicinoid systems biology. *Plant Physiology, 150*(4), 1806-1821.

[24] Medina-Lara, F., Echevarría-Machado, I., Pacheco-Arjona, R., Ruiz-Lau, N., Guzmán-Antonio, A., & Martinez-Estevez, M. (2008). Influence of nitrogen and potassium fertilization on fruiting and capsaicin content in habanero pepper (*Capsicum* chinense Jacq.). *HortScience*, *43*(5), 1549-1554.

[25] Monforte-González, M., Guzmán-Antonio, A., Uuh-Chim, F., & Vázquez-Flota, F. (2010). Capsaicin accumulation is related to nitrate content in placentas of habanero peppers (Capsicum chinense Jacq.). *Journal of the Science of Food and Agriculture*, *90*(5), 764-768.

[26] Moneruzzaman Khandaker, M., Rohani, F., Dalorima, T., & Mat, N. (2017). Effects of Different Organic Fertilizers on Growth, Yield and Quality of *Capsicum Annuum L. Var. Kulai* (Red Chilli Kulai). *Biosciences Biotechnology Research Asia, 14*(1), 185-192.

[27] Moradzadeh, S., Siavash Moghaddam, S., Rahimi, A., Pourakbar, L., & Sayyed, R. Z. (2021). Combined bio-chemical fertilisers ameliorate agro-biochemical attributes of black cumin (Nigella sativa L.). *Scientific Reports*, *11*(1), 11399.

[28] Naves, E. R., de Ávila Silva, L., Sulpice, R., Araújo, W. L., Nunes-Nesi, A., Peres, L. E., & Zsögön, A. (2019). Capsaicinoids: pungency beyond *Capsicum*. *Trends in Plant Science, 24*(2), 109-120.

[29] Ng, T., Ngai, P., Cheung, R., Wong, J., Lam, S., & Wang, H. (2011). Antifungal and mitogenic activities of cluster pepper (*Capsicum frutescens*) Seeds. *Nuts and Seeds in Health and Disease Prevention*, 345-349. Doi: 10.1016/b978-0-12-375688-6.10041-6.

[30] Nguyen, B. T., Harper, S. M., O'Hare, T. J., Menzies, N. W., & Wehr, B. (2022). Sulfur nutrition affects garlic bulb yield and allicin concentration. *Plants, 11*(19), 2571.

[31] Othman, Z. A. A., Ahmed, Y. B. H., Habila, M. A., & Ghafar, A. A. (2011). Determination of capsaicin and dihydrocapsaicin in *Capsicum* fruit samples using high performance liquid chromatography. *Molecules, 16*(10), 8919-8929.

[32] Pascual, I., Azcona, I., Aguirreolea, J., Morales, F., Corpas, F. J., Palma, J. M., ... & Sánchez-Díaz, M. (2010). Growth, yield, and fruit quality of pepper plants amended with two sanitized sewage sludges. *Journal of Agricultural and Food Chemistry*, *58*(11), 6951-6959.

[33] Phocaides, A. (2007). Handbook on pressurized irrigation techniques. *Food & Agriculture Org.*

[34] Rahim, R., & Mat, I. (2012). Phytochemical contents of *Capsicum frutescens* (Chili Padi), *Capsicum annum* (Chili Pepper) and *Capsicum annum* (Bell Peper) aqueous extracts. *International Conference on Biological and life sciences* (Vol. 40, pp. 164-167).

[35] Randviir, E. P., Metters, J. P., Stainton, J., & Banks, C. E. (2013). Electrochemical impedance spectroscopy versus cyclic voltammetry for the electroanalytical sensing of capsaicin utilising screen printed carbon nanotube electrodes. *Analyst, 138*(10), 2970-2981.

[36] Reyes-Escogido, M. D. L., Gonzalez-Mondragon, E. G., & Vazquez-Tzompantzi, E. (2011). Chemical and pharmacological aspects of capsaicin. *Molecules*, *16*(2), 1253-1270.

[37] Saleem, S., Mushtaq, N. U., Rasool, A., Shah, W. H., Tahir, I., & Rehman, R. U. (2023). Plant nutrition and soil fertility: physiological and molecular avenues for crop improvement. In Sustainable Plant Nutrition (pp. 23-49). Academic Press.

[38] Samsuri, S. F. M., Ahmad, R., & Hussein, M. (2010, May). Development of nutrient solution mixing process on time-based drip fertigation system. *2010 Fourth Asia international conference on mathematical/analytical modelling and computer simulation* (pp. 615-619). IEEE.

[39] Silva, A., Binotti, F., Matos, A., Silva, T., Silva, T., & Costa, E. (2021). Priming and growth inhibitor in the production of high-quality pepper seedlings. *Revista De Agricultura Neotropical, 8*(2), e5828.

[40] Suhaimi, M. Y., & Sembok, W. S. W. Z. W. (2021, March). Effect of different nitrogen fertilization levels on plant growth, yield and 6-gingerol content of ginger (*Zingiber officinale*).

[41] Steer, B. (1973). Diurnal variations in photosynthetic products and nitrogen metabolism in expanding leaves. *Plant Physiology, 51*(4), 744-748.

[42] Suhana, O., Norfadzilah, A. F., Mohd Zamri, K., & Siti Nur Hafizah, M. (2019). Crossability and compatibility rate in crosses between semerah chilli (*capsicum* annuum) with selected chilli padi varieties (*capsicum* frutescens). *Transactions of the Malaysian Society of Plant Physiology,* 103.

[43] Supalkova, V., Stavelikova, H., Krizkova, S., Adam, V., Horna, A., & Havel, L. *et al*. (2007). Study of capsaicin content in various parts of pepper fruit by liquid chromatography with electrochemical detection. *12Th International Symposium on Separation Sciences.*

[44] Surapat, W., Pukahuta, C., Rattanachaikunsopon, P., Aimi, T., & Boonlue, S. (2013). Characteristics of phosphate solubilization by phosphate-solubilizing bacteria isolated from agricultural chili soil and their efficiency on the growth of chili (*Capsicum* frutescens L. cv. Hua Rua). *Chiang Mai J Sci., 40*(1), 11-25.

[45] Spence, C. (2018). Why is piquant/spicy food so popular? *International Journal of Gastronomy and Food Science, 12*, 16-21.

[46] Thangasamy, A., Gorrepati, K., Ghodke, P. H., TP, S. A., Jadhav, M., Banerjee, K., & Singh, M. (2021). Effects of sulfur fertilization on yield, biochemical quality, and thiosulfinate content of garlic. *Scientia Horticulturae, 289*, 110442.

[47] Yamamoto, S., & Nawata, E. (2004). Morphological characters and numerical taxonomic study of *Capsicum* frutescens in Southeast and East Asia. *Tropics*, *14*(1), 111-121.

[48] Zhang, Z., Zhao, J., Hu, T., Wang, K., & Yin, Y. (2016). Cloning and functional analysis of the capsaicin synthase gene (Pun1) from pepper (Capsicum chinense). *Journal of Plant Biochemistry and Biotechnology, 25*(4), 382-388.