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The antioxidant content and activity of lemongrass [*Cymbopogon citratus* (DC. ex Nees) Stapf] under the combination of drought stress and nano-silica treatments

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Abstract

This study aimed to determine the effect of a combination of drought stress and nano-silica treatments to increase the content of antioxidant compounds and antioxidant activity of lemongrass [*Cymbopogon citratus* (DC. ex Nees) Stapf] and determine the optimal combination. The research used a Completely Randomized Design (CRD) with two factors with 5 replications. The first factor was the level of drought stress (without drought stress, moderate, and severe). The second factor was the nano-silica dose, namely 0 mg/L, 125 mg/L, and 250 mg/L with a volume of 10 mL per plant. The parameters measured included carotenoid content, proline levels, total phenolic content, and antioxidant activity. Data were analyzed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). The results showed that there is a specific combination of drought stress and nano-silica which can increase the content of carotenoids, proline, total phenolics, and antioxidant activity in lemongrass. The treatment of moderate and severe drought stress with 125 and 250 mg/L nano-silica increased the carotenoid and proline content maximally. Higher total phenol content and antioxidant activity were obtained from the combination of 250 mg/L nano-silica without drought stress. The highest chlorophyll content was recorded from the combination of severe drought stress and 250 mg/L nano-silica. Drought stress treatment with 250 mg/L nano-silica could increase the antioxidants of lemongrass plants, but maximum production of antioxidants required different combinations of drought stress and nano-silica treatments. Nano-silica treatment at a dose of 250 mg/L in conditions of sufficient water or lack of water can be an optimal combination treatment in lemongrass cultivation.

Key words: Antioxidant content, antioxidant activity, *Cymbopogon citratus*, drought stress, lemongrass, nano-silica

Introduction

Lemongrass [*Cymbopogon citratus* (DC. ex Nees) Stapf] belongs to the Poaceae family which has many advantages, such as a cooking spice, detergent, cosmetics, and perfume. Lemongrass is also a plant that has medicinal properties, particularly as an antioxidant, anti-microbial, anti-fungi, and anti-cancer (Oladeji *et al.*, 2019). Several benefits of lemongrass can be acquired from the essential oil content of phytosterols in the form of geraniol, geranial, citronellal, neral, citral, and β-myrrhene (Bayala *et al.*, 2020). Besides, lemongrass also contains secondary metabolites in the form of alkaloids, tannins, flavonoids, phenols, steroids, and saponins (Das *et al.*, 2023).

Degenerative diseases such as cancer, diabetes, and cardiovascular conditions, major global causes of death driven by free radicals, emphasize the critical role of antioxidants in neutralizing these harmful molecules and the need for abundant antioxidant sources in healthcare (Tiwana *et al.*, 2024).

The inflated market demand for the benefits of lemongrass predominantly as a source of antioxidants has encouraged efforts to intensify the productivity and quality of lemongrass with appropriate cultivation innovations. Providing drought stress treatment is one effort that can be made to increase lemongrass antioxidants. Drought stress can increase the total phenols and antioxidant activity of *Oudneya africana* L. (Sihem *et al*, 2020). Drought stress can also increase the synthesis of other antioxidant compounds such as carotenoids and proline in African eggplant (*Solanum aethiopicum* L.) according to Mibei *et al.* (2018).

Drought stress in plants triggers excess reactive oxygen species (ROS), prompting the production of antioxidant compounds to counter oxidative damage (Anjum *et al.*, 2017). According to Li *et al.* (2022), drought stress activates the enzyme phenylalanine ammonia-lyase (PAL), which shifts metabolism from the shikimate pathway (producing primary metabolites) to the phenylpropanoid pathway (producing secondary metabolites). These secondary metabolites play a crucial role in antioxidant defense against environmental stress.

On the other hand, the use of nanosilica in plants can also expand the antioxidant content. The use of silica in wheat (*Triticum aestivum* L.) causes an enhancement in the expression of antioxidant coding genes from the enzyme and non-enzyme groups (Akhtar and Ilyas, 2022). Shi *et al.* (2016) reported that nano-silica also intensified the synthesis and activity of antioxidants in tomatoes (*Solanum lycopersicum*). Silica can reduce plant oxidative stress by regulating the biosynthesis of several antioxidative defense pathways, such as enzymatic antioxidants, the ascorbate-glutathione (ASC-GSH) cycle, and secondary metabolite production. Under environmental stress conditions, silica supplementation helps plants induce the expression of genes directly related to the antioxidative defense pathway (Ma *et al.*, 2015). The potential of providing silica to

plants has great benefits in stimulating the antioxidant content and activity of lemongrass to be higher.

The combination of drought stress and nanosilica treatments to enhance antioxidant content and activity in plants remains underexplored. This study aimed to identify the optimal combination of drought stress and nanosilica for maximizing antioxidant content and activity in lemongrass.

Materials and methods

Equipments and materials: The substantial equipments used were: an oven (Memmert UN 55), scales (Shimadzu AEL 200), rotary evaporator (Bibby Sterilin RE200), water bath (Memmert WNB 22), Uv-Vis spectrophotometer (Shimadzu UV mini 1240), and shaker incubator (Biosan ES-20/60). The substances used were 2 week old lemongrass seeds obtained from the Research and Development Center for Medicinal Plants and Traditional Medicine (B2P2TOOT) Karanganyar, planting media, nanosilica (SiO_2), 25% NaNO, $AlCl_3$, NaOH, 2% Na_2CO_3, 1, 1-diphenyl-picrylhydrazyl (DPPH) 93 µM, sulfo-salicylate 3%, acetone 80%, glacial acetic acid, ninhydrin, toluene, ethanol 70% and 96%.

Preparation and planting: Seed selection was based on the similarity of plant height, number of leaves, and number of tillers, as well as whether the seeds are in fresh condition. The planting medium per pot consisted of a mixture of 1 kg of soil, husks, and compost in a ratio of 2:1:1. Two week-old lemongrass seedlings were transferred into pots, each pot filled with 1 lemongrass seedling.

Nanosilica treatment: Nanosilica treatment was given after the plants were 3 weeks old with doses of 0 mg/L, 125 mg/L, and 250 mg/L, each 10 mL per plant. The treatment was carried out every 2 weeks at 10 mL by spraying it all over the surface of the leaves (Zahedi *et al.*, 2020). Treatment was carried out until the plants were 11 weeks old.

Drought stress treatment: Drought stress treatment began when the plants were 4 weeks old, with daily watering to achieve 100% (no stress), 50% (moderate), and 25% (severe) field capacity, following the method by Darmanti *et al.* (2016). The treatment continued until the plants were 12 weeks old.

Carotenoid content: Analysis of carotenoid content was carried out using the Wellburn method used by Saptiningsih *et al.* (2023). Lemongrass leaves as much as 0.1 g were extracted in 10 mL of acetone (80%). The pigment absorbance was read at wavelengths of 480 nm, 649 nm, 665 nm then analyzed using the formula:

Carotenoids (µmol/g)= (1,000 x A480)-(2.14 x Cl a)-(70.16 x Cl b)/220

where A = absorbance of carotenoid in wavelength of 480 nm, Cl a = chlorophyll a (µmol/g), Cl b = chloropyll b (µmol/g).

Proline content: 50 mg of lemongrass leaves were ground with liquid nitrogen, and homogenized in 10 mL of 3% sulfosalicylic acid, then filtered. 2 mL of filtrate was mixed with about 2 mL of ninhydrin acid and 2 mL of glacial acetic acid. The solution was stored at 100ºC for 60 minutes and the reaction was stopped by placing the solution in an ice bath. A total of 4 mL of toluene was attached to the solution and stirred for 20 seconds. The absorption of the toluene phase was measured at a wavelength of 520 nm (Darmanti *et al.*, 2017).

The proline content was calculated using a proline standard curve. Proline standard solutions were made with several variations in concentration, then the absorption of each solution was measured and a regression equation was created. The absorbance of the lemongrass extract was input into the equation to obtain the proline content of the lemongrass extract.

Total phenol content and antioxidant activity: Extract preparation was carried out for analysis of total phenols and antioxidant activity. Lemongrass fronds were washed and dried, then ground into powder. A total of 250 g of lemongrass stem powder was macerated in 1,000 mL of 70% ethanol for 72 hours with an incubator shaker at room temperature. The maceration results were filtered with filter paper. The extract was then concentrated using a rotary evaporator at a temperature of 35ºC and continued with a waterbath until a paste was formed.

Measurement of total phenol content followed the method of Aalipour *et al.* (2015). A total of 1 mL of lemongrass extract solution with a concentration of 1,000 ppm, 4 mL of 7.5% Na_2CO_3, and 5 mL of Folin-Ciocalteau reagent. Then the solution was diluted with distilled water (1:1) and incubated for 60 minutes at 37°C in dark conditions. Absorbance was measured at a wavelength of 778 nm. Total phenol was determined based on a gallic acid standard curve.

The antioxidant activity test was carried out using the DPPH method used by Magfiroh *et al.* (2023). A total of 0.5 mL of lemongrass extract was reacted with 1.5 mL of DPPH solution (93 µM) in ethanol and stirred for 2 minutes. The efficiency of free radical capture was indicated by the degree of change in color of the solution from purple to yellow with an incubation time of 30 minutes. Absorbance was measured at a wavelength of 515 nm. The percentage of DPPH color reduction is calculated based on the formula.

$$\text{Inhibition} = \frac{\text{Blank absorbance - Sample absorbance}}{\text{Blank absorbance}} \times 100$$

Data analysis: The study used a Completely Randomized Design (CRD) with two factors: drought stress levels (100%, 50%, 25% field capacity) and nanosilica concentrations (0, 125, 250 mg/L at 10 mL/plant). Each treatment had five replicates. Data were analyzed using ANOVA and DMRT in SPSS (Version 25).

Results and discussion

Carotenoid content: The application of nanosilica at all levels of drought stress increased lemongrass carotenoid content. Likewise, at all doses of nanosilica, drought stress led to higher lemongrass carotenoid content. Thus, the application of nanosilica under drought-stress conditions can increase lemongrass carotenoid content. The interaction between drought stress treatment and nanosilica was synergistic towards increasing carotenoid content.

Drought stress induces plant antioxidative defense systems, one of which is carotenoids, compounds to suppress elevated free radicals, such as H_2O_2, to prevent cell damage (Sharma and Zheng, 2019). Nanosilica helps plants in increasing the production of antioxidant compounds (Ma *et al.*, 2016). Increased production of antioxidant compounds due to nanosilica application in lemongrass can suppress free radical activity due to drought stress.

Silica treatment in rose (*Rosa hybrida*) could regulate the transcription of enzymes involved in the ascorbate-glutathione cycle (ASC-GSH) such as glutathione synthetase (GS), glutathione reductase (GR), monodehydascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR), as well as

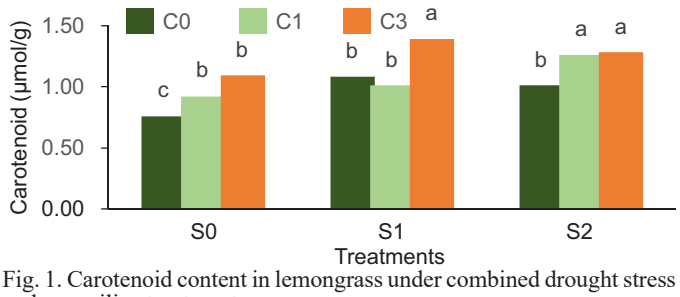

Fig. 1. Carotenoid content in lemongrass under combined drought stress and nanosilica treatments.

in secondary metabolism such as L-phenylalanine ammonia-liase (PAL), chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), and anthocyanin synthase (ANS) (Ma *et al.*, 2016).

Proline content: Lemongrass showed high proline levels under 0 mg/L nanosilica without drought stress, though these were still lower than levels observed with 250 mg/L nanosilica under moderate and severe drought stress. Low proline content typically indicates minimal stress, while higher levels suggest environmental stress affecting plant physiology. Excess water in the soil during the 0 mg/L treatment likely caused inundation stress, prompting proline accumulation to balance water levels and reduce oxidative damage (Kishor *et al.*, 2014).

As a C4 plant, lemongrass adapts well to dry conditions (Rudov *et al.*, 2020), making 100% field capacity unsuitable and stressful. Similarly, the combination of 250 mg/L nanosilica with drought stress triggered high proline content, reflecting activated defense mechanisms. This increase is likely due to excess nanosilica, as lower doses (125 mg/L) under the same drought conditions did not produce similar results.

In addition, lemongrass naturally has a certain amount of silica deposits in its cell wall (Madi *et al.*, 2022). If the supply of exogenous nanosilica is too high, the excess silica will interfere with lemongrass metabolism. Thus, the provision of nanosilica that is too high (250 mg/L) is thought to be responded by

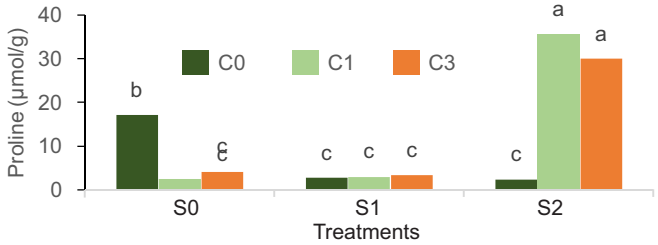

Fig. 2. Proline content in lemongrass under combined drought stress

lemongrass as a form of environmental stress, so that proline production increases. Abbas *et al.* (2015) also stated that the application of nanosilica when salinity stress conditions cause physiological and biochemical changes in plants to stimulate proline synthesis.

Total phenol content: The total phenol content of lemongrass tends to be lower at the level of severe drought stress without nanosilica (0 mg/L dose). The provision of nanosilica at the level of moderate and severe drought stress caused the total phenol content not to be significantly different from the treatment of 0 mg/L nanosilica combination without drought stress. That is, in this case, the addition of nanosilica under water shortage conditions provided the same total phenol content as lemongrass plants without nanosilica application under water-sufficient

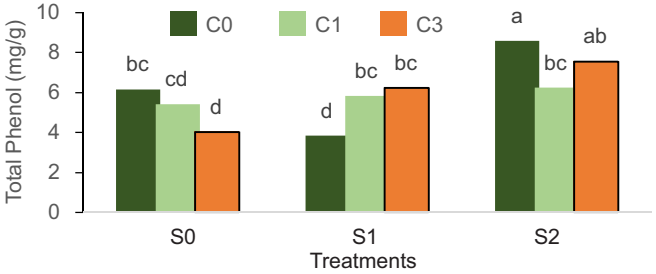

Fig. 3. Total phenolic content in lemongrass under combined drought

conditions. Thus, the addition of nanosilica can help lemongrass produce the same total phenol content under limited water supply conditions. In addition, the application of 250 mg/L nanosilica without drought stress can help increase the total phenol content of lemongrass compared to without nanosilica and 125 mg/L nanosilica.

Nanosilica treatment in drought-stressed plants generally helps increase total phenol content as an antioxidative defense of plants. According to Ma *et al.* (2016), Si application is one of the efforts to increase the content of plant antioxidant compounds by inducing gene expression to increase the accumulation of phenolic compounds (Shetty *et al.*, 2011) and stimulating the synthesis and antioxidant activity in tomato (*Solanum lycopersicum* L.) (Shi *et al.*, 2013).

Antioxidant activity: The combination of drought stress and nanosilica generally had no significant effect on lemongrass antioxidant activity. However, the highest antioxidant activity occurred with 250 mg/L nanosilica under non-drought conditions, likely due to the influence of antioxidant compounds like carotenoids, proline, and phenols. Among these, total phenols appeared to play a major role, as their pattern mirrored antioxidant

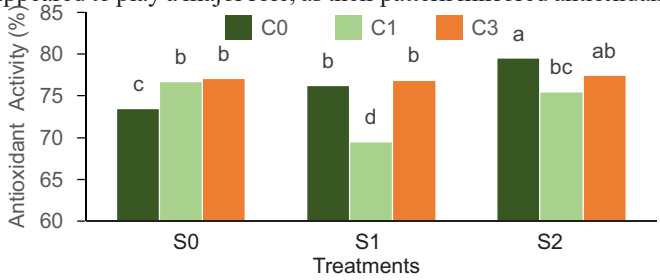

Fig. 4. Antioxidant activity in lemongrass under combined drought stress and nanosilica treatments.

activity levels.

The 250 mg/L nanosilica treatment without drought stress likely induced mild stress, possibly from excess water or high nanosilica concentration, leading to increased ROS. This stress appears to have triggered both phenol production and antioxidant activity, resulting in the highest recorded levels for both.

In lemongrass, variations in antioxidant activity might be linked to the inconsistent increase in antioxidant parameters due to treatment. This aligns with studies on peas (Sutulienė *et al.*, 2021) and roses (Hajizadeh *et al.*, 2023), which found that combining nanosilica with different water levels can enhance plant antioxidant activity.

Lemongrass antioxidants can be increased by combining drought stress treatment with 250 mg/L nanosilica. However, maximum production of different types of antioxidants requires different combinations of drought stress and nanosilica treatments. The

optimal combination of treatments can be adjusted according to the type of antioxidant to be enhanced. Further research can be conducted to find the treatment optimization for increasing specific antioxidant content. Nanosilica treatment at a dose of 250 mg/L under water-sufficient or water-deficient conditions may be the optimal treatment combination in lemongrass cultivation for general antioxidant content.

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