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EFFECT OF SALICYLIC ACID AND ARBUSCULAR MYCORRHIZA FUNGI ON THE GROWTH AND ESSENTIAL OIL PRODUCTION OF CITRONELLA GRASS (*CYMBOPOGON NARDUS* L.)

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ABSTRACT

Salicylic acid (SA) and arbuscular mycorrhizal fungi (AMF) are potential approaches for improving the growth and essential oil production of citronella grass (Cymbopogon nardus L.). This study evaluated the interactive effects of salicylic acid (SA) and arbuscular mycorrhizal fungi (AMF) on citronella growth, physiological traits, and essential oil production using a 4 × 4 factorial Completely Randomized Design with four AMF doses (0, 20, 30, and 40 g plant^{-1}), four SA concentrations (0, 100, 150, and 200 mg L^{-1}), four replicates, and a total of 64 experimental units. Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. The interaction between AMF and SA was associated with increased vegetative growth, particularly leaf number. AMF significantly increased shoot fresh and dry weights, with the highest mean values of 145.94 and 34.93 g plant^{-1}, respectively, at 40 g plant^{-1} AMF. Essential oil yield varied among treatment combinations, with the highest observed yield of 0.105 g plant^{-1} (4.20 kg ha^{-1}) obtained with 200 mg L^{-1} SA combined with 30 g plant^{-1} AMF. The highest numerical oil content (0.072%) was recorded at 100 mg L^{-1} SA without AMF. Essential oil yield was strongly and positively correlated with oil content ($r = 0.884$, $P<0.001$). GC–MS analysis showed that citronellal, citronellol, and geraniol were the predominant constituents, and the selected combined treatment showed a different relative composition than the untreated control. Mycorrhizal colonization reached 55% under the combination of 30 g plant^{-1} AMF and 150 mg L^{-1} SA. The combined application of AMF and SA improved biomass production, physiological performance, and essential oil characteristics, indicating its potential to enhance citronella productivity.

***Keywords*:** Biofertilizer, Volatile compound, Essential oil, Poaceae, Secondary metabolites.

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1. INTRODUCTION

Essential oils (EOs) are valuable secondary metabolites characterized by distinctive organoleptic properties and a wide range of biological activities (Kiani et al., 2022). Their global demand has risen markedly in recent years, largely owing to their increasing use in complementary medicine and their expanding applications across the pharmaceutical, cosmetic, wellness, veterinary, agroecological sectors, food preservation, aromatherapy, and biopesticide industries due to their characteristic aroma and diverse biological activities (Butzge et al., 2023). Furthermore, the production and commercialization of EOs have grown significantly (Nikkhah et al., 2024). Among EO-producing plants, species of the *Cymbopogon* genus (Poaceae) are an important source of bioactive compounds, exhibiting diverse pharmacological and agricultural properties, including antibacterial, insect-repellent, antifungal, anti-inflammatory, larvicidal, and antidiabetic activities (Tibenda et al., 2022). One species in the Cymbopogon genus is Citronella (Cymbopogon nardus L.), an economically important aromatic crop cultivated for its essential oil, which is rich in commercially valuable monoterpenes such as citronellal, citronellol, and geraniol (Bergman et al., 2023). In industrial applications, citronella oil exhibits antioxidant, antibacterial, antiviral, antifungal, antihypercholesterolemic, and anticancer activities, and has also demonstrated potential for biofuel development (Sari et al., 2022; Sugiaman et al., 2024; Ramadhan et al., 2026). Despite its considerable economic importance, citronella

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production is frequently constrained by inconsistent biomass productivity and variations in essential oil yield and quality, thereby limiting its commercial competitiveness and industrial value. Consequently, developing sustainable cultivation strategies that enhance biomass accumulation while optimizing essential oil biosynthesis remains a major challenge (Marcelino et al., 2023)

Plant nutritional status and physiological performance strongly influence essential oil productivity and chemical composition (Khammar et al., 2021). Conventional approaches relying heavily on synthetic fertilizers can improve biomass production but may adversely affect soil health and environmental sustainability (Khan et al., 2024). These concerns have increased the need for environmentally friendly agronomic strategies that can simultaneously enhance plant growth and secondary metabolite biosynthesis (Marks et al., 2025). One promising approach is using beneficial soil microorganisms, which can improve nutrient acquisition and stimulate secondary metabolite production in aromatic and medicinal plants (Wang et al., 2022). Toaima et al. (2023) reported that the application of biological fertilizer increased plant height and total dry weight in summer savory, while also enhancing essential oil content compared with organic. The beneficial effects of improved nutrient availability are closely associated with photosynthetic carbon assimilation, as enhanced nutrient status can increase photosynthetic capacity and the production of photosynthates that serve as carbon precursors for both plant growth and secondary metabolism (Akhter et al., 2025). Through glycolysis and downstream metabolic pathways, these photosynthetic products are converted into amino acids required for biomass accumulation and terpenoids, the principal constituents of essential oils (Fakhimi & Grossman, 2024). Thus, because plant growth and terpenoid biosynthesis depend on a shared carbon pool, the partitioning of photosynthetically derived carbon may play a critical role in determining both biomass accumulation and essential oil production (Raffo et al., 2020).

Within this context, arbuscular mycorrhizal fungi (AMF) have attracted considerable attention as a sustainable biological strategy for improving nutrient acquisition and essential oil production in aromatic plants. In aromatic and medicinal plants such as *Cymbopogon citratus*, AMF inoculation has been recognized as an effective and environmentally sustainable approach for improving plant growth and essential oil yield (Sinha & Kumar, 2026). This beneficial effect is particularly relevant under abiotic stress conditions, as AMF can enhance root architecture, leaf area development, nutrient uptake, and soil quality, thereby improving plant growth and stress tolerance (Farhaoui et al., 2025). Beyond their effects on plant growth and nutrient acquisition, AMF can also stimulate secondary metabolism, leading to greater accumulation of essential oils and phenolic compounds that contribute to the biological activity and commercial value of aromatic plants (Azevedo et al., 2024).

AMF are particularly important because they form symbiotic associations with plant roots, enhancing nutrient acquisition and plant productivity. Through extensive extraradical hyphal networks, AMF improve phosphorus and water uptake, contributing to enhanced plant growth, stress tolerance, and soil fertility (Yilmaz & Karik, 2022). Beyond their role in plant nutrition, AMF have also been reported to modulate secondary metabolism and stimulate the accumulation of essential oils and other bioactive compounds in aromatic crops (Zhao et al., 2022). Moreover, enhanced nutrient acquisition and photosynthetic capacity under AMF symbiosis may promote carbon assimilation and its allocation toward terpene biosynthesis, thereby increasing essential oil yield and influencing its chemical composition (Guo et al., 2025; Li et al., 2025). In addition to biological approaches, chemical elicitors provide a complementary way to stimulate plant defense responses and secondary metabolite biosynthesis, including essential oil production (Ghojavand et al., 2024). Among these elicitors, salicylic acid (SA) is an important signaling molecule involved in regulating photosynthesis, stress responses, and secondary metabolism (Gorni et al., 2020). SA regulates diverse physiological and metabolic processes associated with stress adaptation and secondary metabolite biosynthesis, thereby influencing essential oil production in aromatic plants (Khezri et al., 2023). Exogenous SA application can enhance plant growth and physiological performance while stimulating essential oil biosynthesis and accumulation in aromatic and medicinal plants (Ghassemi-Golezani & Farhadi, 2022). Afkar (2024) reported that SA application increased the accumulation of essential oil constituents in *Mentha piperita*. In addition, SA can modulate the biosynthesis of important classes of secondary metabolites, including alkaloids and terpenes, by regulating specialized metabolic pathways (Kaya et al., 2023; Tadayon et al., 2023).

Moreover, SA plays a pivotal role in regulating secondary metabolite biosynthesis by modulating protein activity and the expression of key genes involved in their respective metabolic pathways (Ding et al., 2023). As a phenolic signaling molecule, SA activates the shikimate pathway and modulates phenylpropanoid metabolism, promoting the accumulation of phenolic compounds, flavonoids, resveratrol, and other bioactive phytochemicals (Kumar et al., 2023). Beyond secondary metabolism, SA regulates a wide range of physiological processes. Beyond secondary metabolism, SA regulates diverse physiological processes, including respiration, photosynthesis, flowering, senescence, growth, and development, which ultimately influence plant productivity (Yu et al., 2022; Negi et al., 2023). These multifaceted functions suggest that SA may complement the effects of AMF by coordinating plant physiological responses and secondary metabolism alongside AMF-mediated improvements in nutrient acquisition (Gorni et al., 2020).

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Although the individual roles of AMF and SA in promoting plant growth and secondary metabolism have been widely documented, their interactive effects remain largely unexplored, particularly in *Cymbopogon nardus*. Integrating AMF and SA represents a promising strategy for enhancing citronella productivity by simultaneously improving nutrient acquisition, physiological performance, and essential oil biosynthesis. Understanding the combined influence of these two factors is essential for developing sustainable cultivation systems that can improve both essential oil yield and chemical quality. Therefore, this study evaluated the combined effects of AMF and SA on plant growth, physiological responses, essential oil productivity, and oil composition of citronella (*Cymbopogon nardus* L.), with particular emphasis on their interaction in regulating biomass accumulation and essential oil biosynthesis.

2. MATERIALS AND METHODS

2.1. Site and Experimental Period

The experiment was conducted in the Greenhouse of the Faculty of Agriculture, Universitas Sebelas Maret, at 170 m above sea level (7°37'48.82" S, 110°56'52.17" E) (Fig. 1). Plant cultivation was conducted for 14 weeks after planting (December–March 2025), followed by postharvest evaluation and subsequent laboratory analyses. During the cultivation phase, environmental conditions such as temperature, relative humidity, and light intensity were recorded using a thermo-hygrometer and lux meter (Fig. 2). The experiment used ultisol soil with chemical properties by pH 6.3, N 0.23%; P 18.77 ppm; K 0.33 %; C Organic 0.27%; Organic matter 0.47%; C/N ratio 1.18.

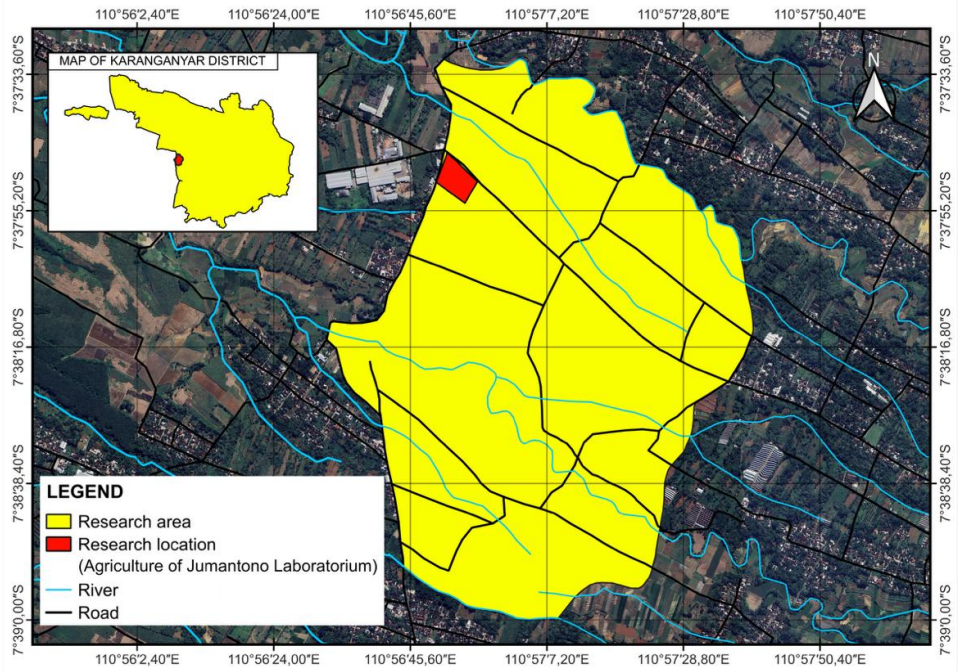

Fig. 1: Location of the experimental site in Jumantono District, Karanganyar Regency, Central Java, Indonesia.

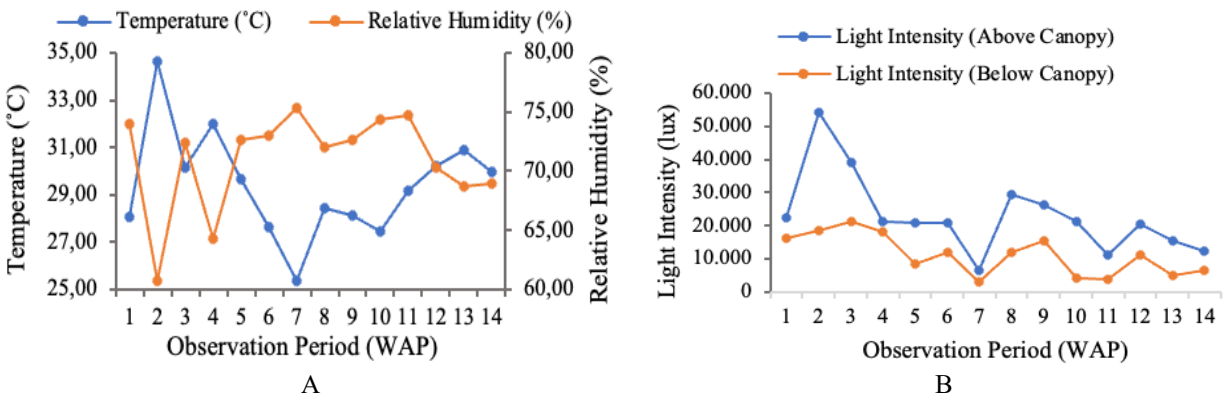

Fig. 2: Temperature and relative humidity recorded in the greenhouse during the observation period (A); Light intensity at above and below canopy during the observation period (B).

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2.2. Materials and Equipment

Vegetative planting materials of citronella (*Cymbopogon nardus* L., cv. Lenabatu) were obtained from one-year-old mother clumps cultivated at the Indonesian Research Center for Medicinal and Aromatic Plants (B2P2TOOT), Tawangmangu, Central Java, Indonesia. To ensure uniformity, the roots of each cutting were trimmed to approximately 10 cm from the stem base, while the leaves were cut to approximately 20 cm above the leaf sheath (Chepyala et al., 2025). Ultisol soil was used as the planting medium. Salicylic acid (SA), arbuscular mycorrhizal fungi (AMF) inoculum, and analytical-grade reagents were used throughout the experiment. Environmental conditions were monitored using a lux meter and a thermo-hygrometer. Chlorophyll content was determined using a UV–Visible spectrophotometer (Genesys 150, Thermo Scientific, USA). Essential oil extraction was performed using a hydrodistillation apparatus, and the chemical composition of the essential oil was analyzed using a Gas Chromatography–Mass Spectrometry (GC–MS) system (GCMS-QP2010, Shimadzu, Kyoto, Japan).

2.3. Experimental Design and Layout

The experiment followed a 4 × 4 factorial Completely Randomized Design (CRD) with two experimental factors. The first factor was salicylic acid (SA) concentration at four levels (0, 100, 150, and 200 mg L^{-1}), and the second factor was arbuscular mycorrhizal fungi (AMF) inoculation at four levels (0, 20, 30, and 40 g $plant^{-1}$). The resulting 16 treatment combinations were replicated four times, giving a total of 64 experimental units. The evaluated variables included the number of leaves, number of tillers, chlorophyll *a*, chlorophyll *b*, total chlorophyll, shoot fresh weight, shoot dry weight, essential oil yield, essential oil content, essential oil composition, and mycorrhizal colonization.

2.4. Planting Medium and Mycorrhizal Inoculation

Each experimental unit consisted of one citronella (*Cymbopogon nardus* L. cv. Lenabatu) cutting planted individually in a 45 × 45 cm polybag filled to approximately three-quarters of its total volume with Ultisol soil. Prior to planting, a consortium of arbuscular mycorrhizal fungi (AMF), comprising *Glomus claroideum*, *Acaulospora rugosa*, *Acaulospora colossica*, *Glomus fasciculatum*, *Glomus mosseae*, and *Glomus etunicatum*, with a total spore density of 98 spores per 100 g of inoculum, was applied by placing the inoculum approximately 2 cm below the root zone according to the respective treatment (Parapasan & Gusta, 2014). Four AMF doses were evaluated: 0 (M0), 20 (M1), 30 (M2), and 40 g $plant^{-1}$ (M3). One citronella cutting was transplanted into each polybag, and the polybags were arranged at a spacing of 50 × 50 cm. The plant density was calculated based on the 50 × 50 cm spacing, corresponding to 40,000 plants ha^{-1} (10,000 m^2 ha^{-1} / 0.25 m^2 $plant^{-1}$). Essential oil yield per hectare was calculated by multiplying the essential oil yield per plant by the estimated plant density. Furthermore, following transplanting, all plants were irrigated daily, either in the morning or afternoon, throughout the experimental period (1–14 weeks after planting, WAP). Irrigation was maintained at 100% field capacity using a watering can. Field capacity was determined gravimetrically by measuring the difference between the weight of saturated and unsaturated planting media (George Michael et al., 2022; Liyanage et al., 2022).

2.5. Salicylic Acid Application

Salicylic acid (SA) solutions were prepared in distilled water to obtain final concentrations of 0 (A0), 100 (A1), 150 (A2), and 200 mg L^{-1} (A3). The solutions were applied as foliar sprays in the morning according to the respective treatment (Gorni et al., 2020). The first application was performed at 30 days after planting (DAP), followed by four subsequent applications at 14-day intervals, corresponding to 44, 58, 72, and 86 DAP (Choudhary et al., 2021). Thus, each plant received a total of five foliar applications throughout the experimental period.

2.6. Chlorophyll Content

Chlorophyll content was determined following Arnon (1949) as adapted by Kurniawan et al. (2021). Leaf samples of citronella grass (0.5 g) were ground in 10 mL of 80% acetone, filtered, and the extract transferred to a glass vial. Absorbance was measured at 645 nm and 663 nm using a UV-Visible Spectrophotometer (Genesys 150, Thermo Scientific), with solvent transmittance set to 100%. Chlorophyll a, b, and total chlorophyll (mg g^{-1}) were calculated according to Arnon (1949):

$$\text{Chlorophyll a} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times (10\ \text{mL}/(1000 \times 0.5\ \text{g}))$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times (10\ \text{mL}/(1000 \times 0.5\ \text{g}))$$

$$\text{Total Chlorophyll} = (20.2 \times A_{645} + 8.02 \times A_{663}) \times (10\ \text{mL}/(1000 \times 0.5\ \text{g}))$$

Where A663 is the absorbance at 663 nm, and A_{645} is the absorbance at 645 nm.

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2.7. Essential Oil Extraction and Determination

Fresh shoot biomass harvested from each experimental unit was cut into 3–5 cm segments, and a 100 g fresh sample was subjected to hydrodistillation with 4 L of distilled water at 100°C for 4 h to extract the essential oil. The extracted essential oil was separated from the distillate, dried over anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture, and weighed. Essential oil yield was expressed as the weight of purified essential oil obtained from each experimental unit (g plant^{-1}). Essential oil content (%) was calculated on a fresh-weight basis using the following equation:

$$Essential\ oil\ content\ (\%) = \frac{Weight\ of\ essential\ oil\ obtained\ (g)}{Fresh\ sample\ weight\ (g)} \times 100$$

2.8. Gas Chromatography–Mass Spectrometry (GC-MS) Analytical Method

Ion source and interface temperatures were 210 and 260°C, respectively. Injection was performed in split mode (split ratio 1:29.9) at 250°C with a pressure of 13.5 kPa. The total gas flow was 79.0 mL/min, and detection was conducted in full scan mode (m/z 28–600) using Electron Ionization (EI) at 70 eV. Identified compounds were reported with their relative percentage composition.

The chemical composition of the essential oil was analyzed only for the control treatment (A0M0) and the treatment with the highest essential oil yield (A3M2) using a Shimadzu GCMS-QP2010 system at Universitas Gadjah Mada, Indonesia. Chromatographic separation was performed on an Agilent DB-5MS UI capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) using helium as the carrier gas at a constant flow rate of 0.25 mL min^{-1}. The oven temperature was initially maintained at 50°C for 5 min, increased to 240°C at a rate of 5°C min^{-1}, and held for 17 min. The injector temperature was set at 250°C with a split ratio of 1:29.9 and an inlet pressure of 13.5 kPa. The total carrier gas flow was 79.0 mL min^{-1}. The ion source and interface temperatures were maintained at 210°C and 260°C, respectively. Mass spectra were acquired in full-scan mode over an m/z range of 28–600 using electron ionization (EI) at 70 eV. Compounds were tentatively identified by comparing their mass spectra with the NIST62 and WILEY229 mass spectral libraries. Compound identification was based on the highest Similarity Index (SI) obtained from the library search, and the relative abundance of each constituent was expressed as the percentage of the total chromatographic peak area.

2.9. Mycorrhizal Colonization Assessment

Mycorrhizal colonization was determined by the presence of mycorrhizal structures within the roots, which were clearly visualized following chemical staining. Root segments (1–2 cm, 25 pieces per treatment) were cleared in 10% KOH at 90°C until softened, rinsed with distilled water, and immersed in 1 N HCl for 5–10 min. Roots were then stained with 0.05% lactophenol trypan blue for 24 hours at room temperature. Twenty stained root segments were mounted on microscope slides and observed under a compound microscope. Root segments exhibiting hyphae, arbuscules, or vesicles were considered colonized (Xiao et al., 2023a). The percentage of AMF colonization was calculated as:

$$AMF\ colonization\ (\%) = \frac{Number\ of\ colonized\ root\ segments}{Total\ number\ of\ observed\ root\ segments} \times 100$$

Colonization intensity was classified as very low (<5%), low (6–25%), moderate (26–50%), high (51–75%), and very high (>75%) according to Rajapakse and Miller (1992).

2.10. Data Analysis

Each experimental unit consisted of one citronella plant grown individually in a polybag. The experiment comprised 16 treatment combinations with four replicates, resulting in a total of 64 experimental units. Environmental parameters, essential oil yield, essential oil content, essential oil constituents identified by GC–MS, and mycorrhizal colonization were analyzed descriptively. Growth parameters (plant height, number of leaves, and number of tillers), physiological parameters (chlorophyll a, chlorophyll b, and total chlorophyll), and biomass yield parameters (shoot fresh weight, shoot dry weight, and root dry weight) were subjected to two-way analysis of variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$). When significant treatment effects were detected, mean separation was performed using Duncan's Multiple Range Test (DMRT) at the 95% confidence level. Pearson correlation analysis was conducted to evaluate the relationships between biomass yield, essential oil yield, and essential oil content using 64 replicate observations. Regression analysis was performed to examine the relationships between the treatment factors and the measured growth, physiological, and biomass yield parameters. Data are presented as mean ± standard deviation (X ± SD).

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3. RESULTS

3.1. Analysis of Variance (ANOVA)

***3.1.1. Number of Leaves*:** A significant SA × AMF interaction was detected for leaf production (Fig. 3; Table 1). Considering all treatment combinations, mean leaf number ranged from 48.63 to 61.13 leaves plant^{-1}, with the maximum value occurring at 200 mg L^{-1} SA in combination with either 0 or 20 g plant^{-1} AMF. Notably, increasing SA concentration did not produce a uniform response across AMF levels. The quadratic regression models (Fig. 4) further demonstrated that the relationship between SA concentration and leaf number varied with AMF inoculation, with R^2 values of 0.5103, 0.9192, 0.9861, and 0.9691 at 0, 20, 30, and 40 g plant^{-1} AMF, respectively. Thus, leaf production exhibited an AMF-dependent response to SA concentration under the experimental conditions.

Table 1: Summary of two-way ANOVA for growth, physiological, and biomass yield parameters of citronella under salicylic acid (SA) and arbuscular mycorrhizal fungi (AMF) treatments

Trait	SA (F, p)	AMF (F, p)	SA × AMF (F, p)
Number of leaves	1.277 (0.293)	1.284 (0.291)	2.433 (0.023) *
Number of tillers	0.612 (0.610)	1.287 (0.290)	1.932 (0.070)
Chlorophyll b	2.569 (0.065)	0.251 (0.860)	0.853 (0.572)
Total chlorophyll	2.546 (0.067)	0.311 (0.817)	1.042 (0.422)
Fresh shoot weight	0.568 (0.638)	7.500 (<0.001) *	1.407 (0.212)
Dry shoot weight	0.544 (0.654)	10.324 (<0.001) *	0.780 (0.635)

Values are F-statistics with the corresponding P-values shown in parentheses. Statistical significance was determined at α = 0.05. *P<0.05.

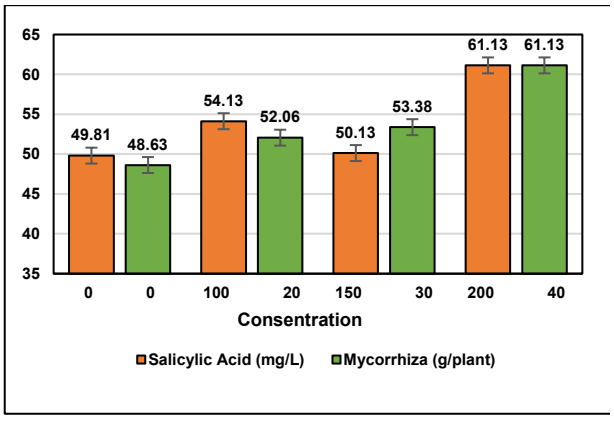

Fig. 3: Effects of Salicylic Acid and Mycorrhizal Inoculation on the Number of Leaves of Citronella Grass.

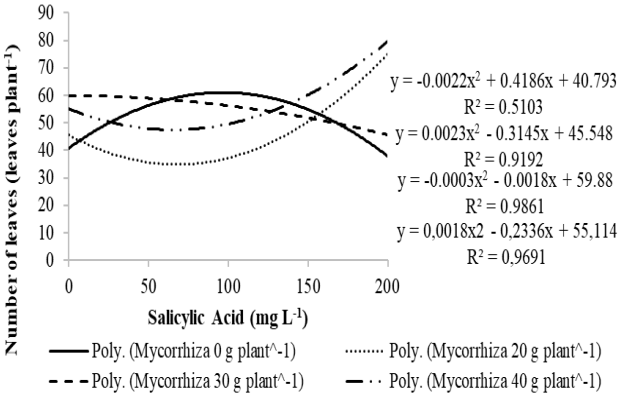

Fig. 4: Regression analysis of the number of leaves in citronella grass.

3.2. Number of tillers

The number of tillers varied numerically across the tested SA concentrations and AMF inoculation levels, although the main effects of SA and AMF and their interaction were not statistically significant (Fig. 5; Table 1). Mean tiller number ranged from 7.63 to 9.25 tillers plant^{-1} across the SA concentrations, with the highest numerical value recorded at 200 mg L^{-1} SA. Furthermore, mean tiller number ranged from 7.00 to 9.63 tillers plant^{-1} across the AMF inoculation levels, with the highest numerical value observed at 40 g plant^{-1} AMF. These numerical patterns indicate a tendency toward greater tiller production at the higher levels of SA and AMF tested, although the observed variation was not sufficient to demonstrate a statistically detectable treatment effect under the experimental conditions.

3.3. Chlorophyll Content

Fig. 7 and Table 1 showed numerical variation in chlorophyll *b* and total chlorophyll contents among the treatment combinations; however, these differences were not statistically significant. The highest mean values were recorded at 200 mg L^{-1} SA, while those at 150 mg L^{-1} SA were comparable. Compared with the untreated control, chlorophyll *b* and total chlorophyll contents at 150 mg L^{-1} SA were numerically higher by 52.17 and 20.97%, respectively; however, these differences were not statistically significant. Overall, chlorophyll accumulation in citronella plants remained relatively stable across the tested SA concentrations and AMF inoculation levels under the experimental conditions.

3.4. Fresh Shoot Weight

Fresh shoot weight was significantly affected by AMF inoculation, whereas the main effect of SA and the

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SA × AMF interaction were not significant (Fig. 8; Table 1). Mean fresh shoot weight increased with increasing AMF inoculation, with the highest value recorded at 40 g plant^{-1} AMF (145.94 g). However, the interaction between SA and AMF was not significant, the biomass response was interpreted primarily in relation to the main effect of AMF rather than indi/vidual SA × AMF treatment combinations. Regression analysis further showed a positive quadratic relationship between AMF dose and fresh shoot weight (Fig. 9), although the quadratic regression model explained only 25.8% of the variation ($R^2 = 0.258$). These results indicate that AMF inoculation contributed to shoot biomass production, while variation in SA concentration did not significantly modify this response under the experimental conditions.

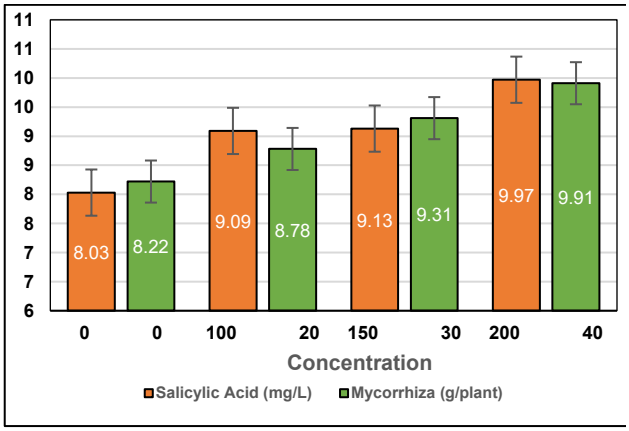


Fig. 5: Effects of Salicylic Acid and Mycorrhizal Inoculation on the Number of Tillers of Citronella Grass.

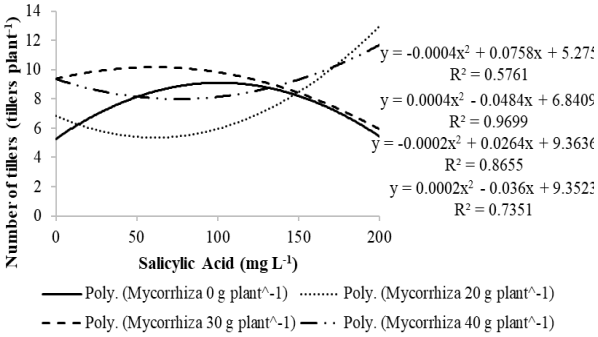
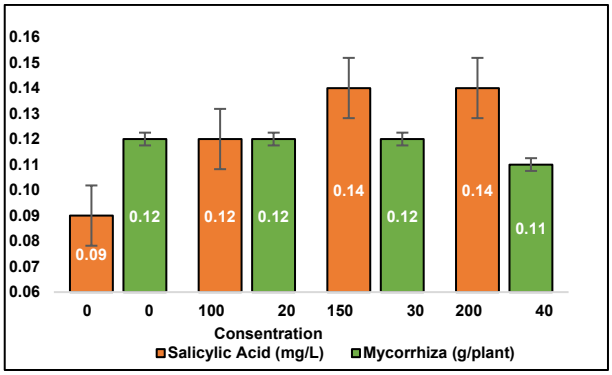
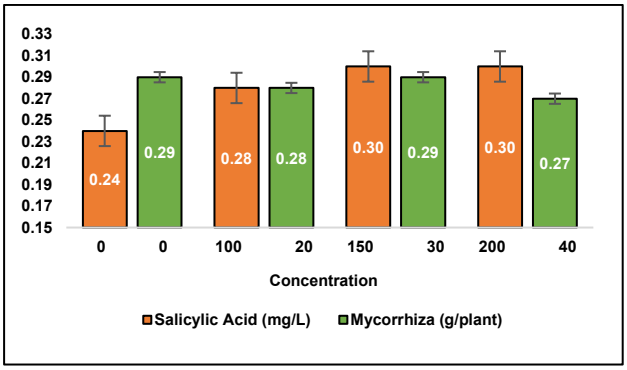


Fig. 6: Regression analysis of the number of tillers in citronella grass.



A



B

Fig. 7: Effects of salicylic acid and mycorrhizal inoculation on A) chlorophyll *b*, and B) total chlorophyll contents of citronella grass.

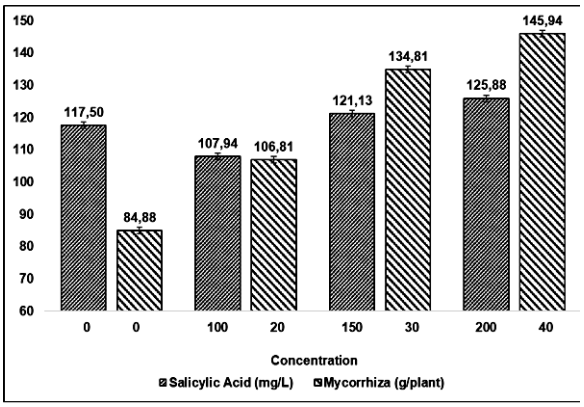


Fig. 8: Effects of Salicylic Acid and Mycorrhizal Inoculation on the Fresh Shoot Weight of Citronella Grass.

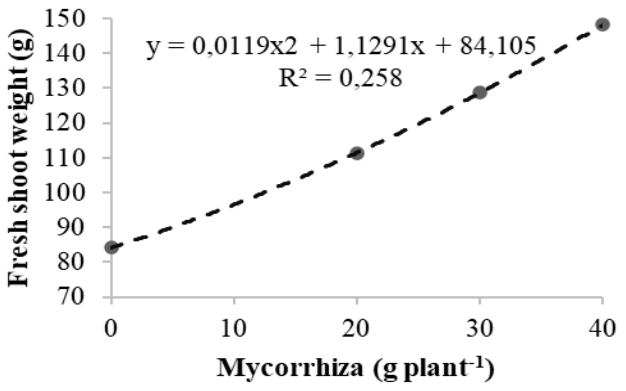


Fig. 9: Regression analysis of the fresh shoot weight in citronella grass.

Citation: Setyawati, A., Faridah, A., Budaraga, I.K., Syukri, D., Saskya, N., & Wijati, L.W. (2026). Effect of salicylic acid and arbuscular mycorrhiza fungi on the growth and essential oil production of citronella grass (*Cymbopogon nardus* L.). Agrobiological Records, 25, 371-387. https://doi.org/10.47278/journal.abr/2026.071

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3.5. Dry Shoot Weight

Dry shoot weight was significantly affected by AMF inoculation, whereas the main effect of SA and the SA × AMF interaction were not significant (Fig. 10; Table 1). Shoot dry weight showed an overall increasing trend with increasing AMF inoculation, with the highest mean value observed at 40 g plant^{-1} AMF (34.93 g). Although the highest numerical value was recorded for the treatment combination of 200 mg L^{-1} SA and 40 g plant^{-1} AMF, the absence of a significant SA × AMF interaction indicates that this combination should not be interpreted as statistically superior. Regression analysis further indicated a positive quadratic relationship between AMF dose and shoot dry weight (Fig. 11), with the model accounting for 34.8% of the observed variation ($R^2 = 0.348$). These findings indicate that AMF inoculation was the primary treatment factor associated with shoot dry biomass, whereas variation in SA concentration did not significantly alter this response under the experimental conditions.

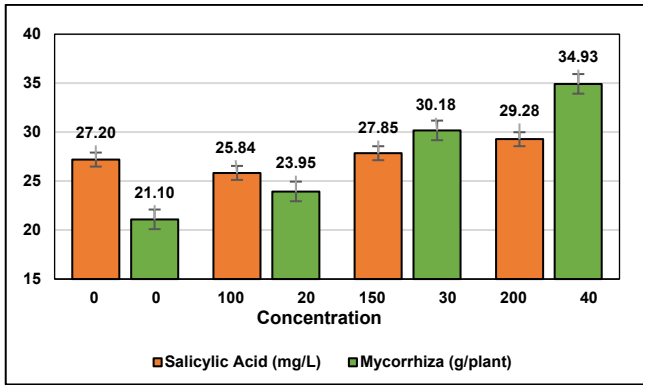

Fig. 10: Effects of Salicylic Acid and Mycorrhizal Inoculation on the Dry Shoot Weight of Citronella Grass.

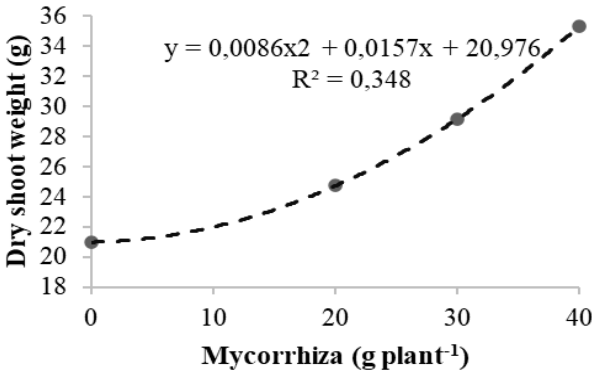

Fig. 11: Regression analysis of the dry shoot weight in citronella grass.

3.6. Essential Oil Yield and Content

Two key parameters in evaluating essential oil production are oil yield (g) and oil content (%). Correlation analysis was performed to examine the relationships between plant growth variables, such as shoot fresh weight and shoot dry weight, with oil yield and oil content (Table 2). Pearson correlation analysis revealed a strong positive and significant relationship between essential oil yield and oil content ($r = 0.884$, $P<0.001$; Table 2). Shoot fresh weight and shoot dry weight were also strongly and significantly correlated ($r = 0.916$, $P<0.001$). However, the correlations of oil yield with shoot fresh weight ($r = 0.472$, $p = 0.065$) and shoot dry weight ($r = 0.489$, $p = 0.055$) were not statistically significant. Similarly, oil content was not significantly correlated with shoot fresh weight ($r = 0.060$, $p = 0.826$) or shoot dry weight ($r = 0.116$, $p = 0.668$).

Table 2: The Pearson correlation results between essential oil yield, oil content, and growth variables

Variable	Shoot Fresh Weight	Shoot Dry Weight	Oil Content
Oil Yield	r = 0.472 (p=0.065)	r = 0.489 (p=0.055)	r = 0.884 (p<0.001)
Shoot Fresh Weight		r = 0.916 (p<0.001)	r = 0.060 (p=0.826)
Shoot Dry Weight			r = 0.116 (p=0.668)

r = Pearson correlation coefficient, p = p-value (level of significance).

Essential oil yield varied among the SA and AMF treatment combinations (Fig. 12). The highest observed yield was recorded under A3M2 (200 mg L^{-1} SA + 30 g plant^{-1} AMF), reaching 0.105 g plant^{-1}, equivalent to 4.20 kg ha^{-1} based on a planting density of 40,000 plants ha^{-1} derived from the 50 × 50 cm spacing. A relatively high yield was also observed under A0M3 (40 g plant^{-1} AMF without SA), at 0.082 g plant^{-1} or 3.28 kg ha^{-1}. In contrast, the lowest observed yields were recorded under A0M1 and A0M2, at 0.003 and 0.006 g plant^{-1}, respectively. Oil content also varied among the treatment combinations (Fig. 12). The highest observed oil content was recorded under A1M0 (100 mg L^{-1} SA without AMF), reaching 0.072%, followed by A1M1 (100 mg L^{-1} SA + 20 g plant^{-1} AMF) and A3M2 (200 mg L^{-1} SA + 30 g plant^{-1} AMF), with values of 0.066% and 0.062%, respectively. In contrast, A3M3 (200 mg L^{-1} SA + 40 g plant^{-1} AMF) showed a relatively low oil content of 0.027%, despite A3M2 producing the highest observed oil yield. Thus, the treatment combination associated with the highest oil yield differed from that producing the highest oil content. Representative plant appearance under the untreated control (A0M0) and A3M2, the treatment combination with the highest observed oil yield, is shown in Fig. 13.

Citation: Setyawati, A., Faridah, A., Budaraga, I.K., Syukri, D., Saskya, N., & Wijati, L.W. (2026). Effect of salicylic acid and arbuscular mycorrhiza fungi on the growth and essential oil production of citronella grass (*Cymbopogon nardus* L.). Agrobiological Records, 25, 371-387. https://doi.org/10.47278/journal.abr/2026.071

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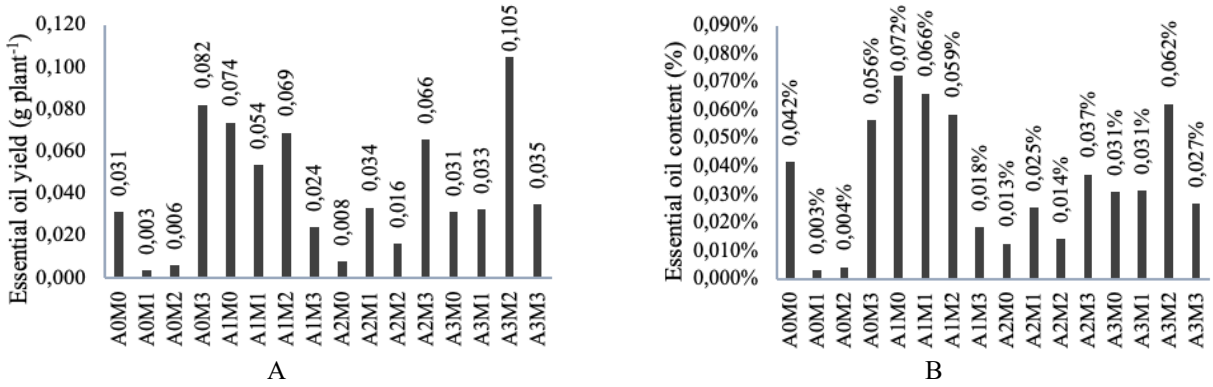

Fig. 12: (A) Essential oil yield and (B) essential oil content of citronella grass (*C. nardus*). A0 = without SA, A1 = SA 100 mg L^{-1}, A2 = SA 150 mg L^{-1}, A3 = SA 200 mg L^{-1}, M0 = without mycorrhiza, M1 = 20 g $plant^{-1}$, M2 = 30 g $plant^{-1}$, M3 = 40 g $plant^{-1}$.

Fig. 13: Appearance of citronella grass biomass under (A) the control treatment (A0M0) and (B) the treatment with the highest total essential oil yield (A3M2).

3.7. Essential Oil Constituents

GC–MS analysis was conducted to characterize the essential oil composition of citronella grass (*C. nardus*) in the untreated control (A0M0) and the selected A3M2 treatment (200 mg L^{-1} SA + 30 g $plant^{-1}$ AMF), which produced the highest observed essential oil yield (Fig. 14). Compound identification was based on comparison of the acquired mass spectra with entries in the Wiley NIST library. The chromatogram of the control sample showed 26 detected peaks, indicating the presence of multiple volatile constituents.

Chemical composition analysis of citronella grass (*C. nardus*) essential oil was performed using GC–MS, and compound identification was based on mass spectra comparison with the Wiley and NIST libraries. The GC–MS chromatogram of the control treatment showed 24 peaks, whereas the combined treatment of SA at 200 mg L^{-1} and mycorrhiza at 30 g $plant^{-1}$, which produced the highest oil yield, displayed 26 peaks (Fig. 14).

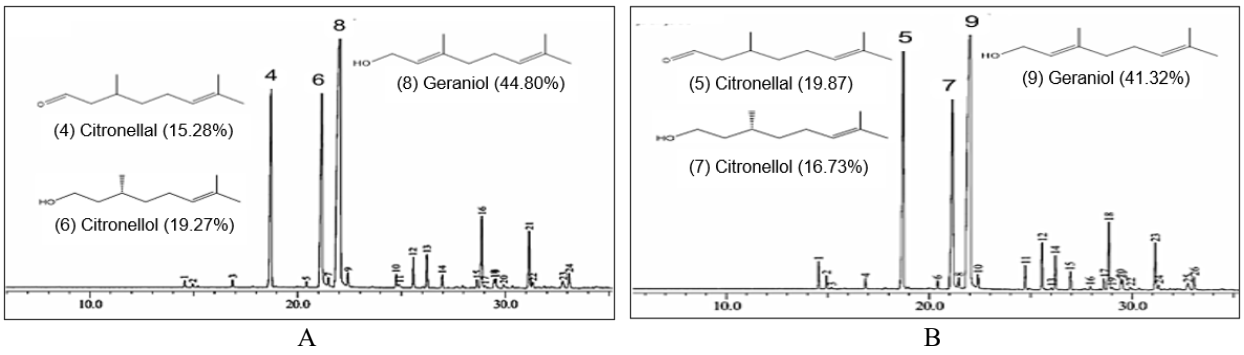

Fig. 14: Chromatogram of citronella grass (*C. nardus*) essential oil from (A) the untreated control (A0M0) and (B) A3M2 (200 mg L^{-1} SA + 30 g $plant^{-1}$ AMF), the treatment with the highest observed essential oil yield.

Citronellal, citronellol, and geraniol were the major constituents in the essential oil of both samples (Table 3). In the untreated control, their relative abundances were 15.28, 19.27, and 44.80%, respectively. In the selected A3M2 treatment, citronellal accounted for 19.87% of the relative peak area, whereas citronellol and geraniol represented 16.73 and 41.32%, respectively. Thus, compared with the control, the A3M2 treatment showed an increase in the relative abundance of citronellal by 4.59 percentage points, accompanied by decreases of 2.54 and 3.48 percentage points in citronellol and geraniol, respectively.

Several minor constituents, including α-pinene, citronellyl acetate, geranic acid, and trans-caryophyllene, were also detected, with differences in their relative abundances between the two samples (Table 3). Overall, the selected A3M2 treatment was associated with a different relative profile of major and minor volatile constituents compared with the untreated control.

Citation: Setyawati, A., Faridah, A., Budaraga, I.K., Syukri, D., Saskya, N., & Wijati, L.W. (2026). Effect of salicylic acid and arbuscular mycorrhiza fungi on the growth and essential oil production of citronella grass (*Cymbopogon nardus* L.). Agrobiological Records, 25, 371-387. https://doi.org/10.47278/journal.abr/2026.071

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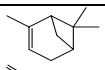
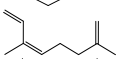
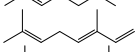
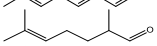
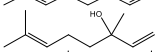
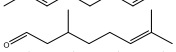
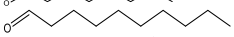
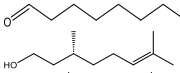
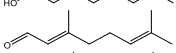
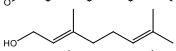
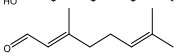
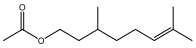
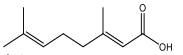
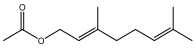
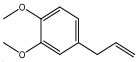
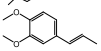
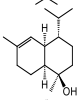
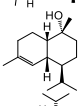
Table 3: Essential oil constituents of citronella grass (*C. nardus*) under the control treatment (A0M0) and the treatment with the highest total essential oil yield (A3M2)

Compound	Molecul Name	Structure	Retention Time (min)		Area (%)		Identification Basis	Change
			A0M0	A3M2	A0M0	A3M2		
α-Pinene	$C_{10}H_{16}$		14.56	14.56	0.36	1.26	WILEY Lib.	↑
α-Ocimene	$C_{10}H_{16}$		14.94	-	0.17	-	WILEY Lib.	↓
trans β-ocimene	$C_{10}H_{16}$		-	14.94	-	0.62	WILEY Lib.	↑
Melonal	$C_9H_{16}O$		-	15.19	-	0.10	WILEY Lib.	↑
Linalool	$C_{10}H_{18}O$		16.87	16.86	0.42	0.52	WILEY Lib.	↑
Citronellal	$C_{10}H_{18}O$		18.73	18.75	15.28	19.87	WILEY Lib.	↑
Decanal	$C_{10}H_{20}O$		20.43	20.42	0.32	0.42	WILEY Lib.	↑
Citronellol	$C_{10}H_{20}O$		21.17	21.16	19.27	16.73	WILEY Lib.	↓
Citral	$C_{10}H_{16}O$		21.48	21.47	0.60	0.70	NIST Lib.	↑
Geraniol	$C_{10}H_{18}O$		22.06	22.04	44.80	41.32	WILEY Lib.	↓
Z-Citral	$C_{10}H_{16}O$		22.42	22.41	0.88	0.79	WILEY Lib.	↓
Citronellyl acetate	$C_{12}H_{22}O_2$		24.75	24.74	0.76	1.17	WILEY Lib.	↑
Geranic Acid	$C_{10}H_{16}O_2$		24.90	25.57	0.30	2.38	WILEY Lib.	↑
β-elemene	$C_{15}H_{24}$		-	26.04	-	0.10	WILEY Lib.	↑
Geranyl acetate	$C_{12}H_{20}O_2$		25.57	-	1.60	-	WILEY Lib.	↓
Methyleugenol	$C_{11}H_{14}O_2$		26.24	26.23	1.94	1.89	WILEY Lib.	↓
Trans-Caryophyllene	$C_{15}H_{24}$		26.97	26.97	0.77	1.04	WILEY Lib.	↑
α-caryophyllene	$C_{15}H_{24}$		-	27.95	-	0.19	WILEY Lib.	↑
Germacrene-D	$C_{15}H_{24}$		28.64	28.63	0.51	0.63	WILEY Lib.	↑
Methyl cis-isoeugenol	$C_{11}H_{14}O_2$		28.88	28.87	4.82	4.28	WILEY Lib.	↓
α-muurolene	$C_{15}H_{24}$		-	29.03	-	0.18	WILEY Lib.	↑
δ-Cadinol (torreyol)	$C_{15}H_{26}O$		29.03	32.76	0.17	0.64	WILEY Lib.	↑
γ-Muurolene	$C_{15}H_{24}$		29.46	29.46	0.53	3.32	WILEY Lib.	↑
δ-Cadinene	$C_{15}H_{24}$		-	29.54	-	0.57	WILEY Lib.	↑
Copaene	$C_{15}H_{24}$		-	29.92	-	0.16	NIST Lib.	↑
Aromadendr-1-ene	$C_{15}H_{24}$		29.55	-	0.82	-	WILEY Lib.	↓
Germacrene D-4-OL	$C_{15}H_{26}O$		31.16	-	3.56	-	WILEY Lib.	↓
Caryophyllene Oxide	$C_{15}H_{24}$		31.33	31.33	0.44	0.32	WILEY Lib.	↓
1β-Cadin-4-en-10-ol	$C_{15}H_{26}O$		32.77	-	0.72	-		↓
α-Cadinol	$C_{15}H_{26}O$		33.08	33.07	0.99	0.81	WILEY Lib.	↓

Citation: Setyawati, A., Faridah, A., Budaraga, I.K., Syukri, D., Saskya, N., & Wijati, L.W. (2026). Effect of salicylic acid and arbuscular mycorrhiza fungi on the growth and essential oil production of citronella grass (*Cymbopogon nardus* L.). Agrobiological Records, 25, 371-387. https://doi.org/10.47278/journal.abr/2026.071

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3.8. Mycorrhizal Colonization

Mycorrhizal colonization in citronella grass roots was absent in all treatments without arbuscular mycorrhizal fungi (AMF) inoculation (M0) (Table 4, Fig. 15). Inoculation with AMF, either alone or in combination with salicylic acid (SA), markedly increased root colonization. Based on the average response across SA treatments, colonization increased from 31% at 20 g plant^{-1} AMF to 43% at 30 g plant^{-1} AMF, reaching 51% at 40 g plant^{-1} AMF (Table 4). At the individual treatment level (Fig. 15), the highest colonization (55%) was recorded in the A0M3 (0 mg L^{-1} SA + 40 g plant^{-1} AMF) and A2M3 (150 mg L^{-1} SA + 40 g plant^{-1} AMF) treatments. The A1M3 treatment (100 mg L^{-1} SA + 40 g plant^{-1} AMF) exhibited 50% colonization, whereas A3M3 (200 mg L^{-1} SA + 40 g plant^{-1} AMF) showed 45% colonization. Microscopic observations further confirmed successful AMF colonization through the presence of vesicular structures within the root cortex, with a greater abundance of vesicles observed as the AMF inoculation dose increased (Fig. 16).

Table 4: Effect of salicylic acid and mycorrhiza on mycorrhizal colonization (%) in citronella grass (*C. nardus*)

Treatment	Mycorrhizal Colonization	
SA (mg L^{-1})	Percentage (%)	Classification
0	34	Moderate
100	29	Moderate
150	31	Moderate
200	31	Moderate
Mycorrhiza (g plant^{-1})		
0	0	(Control)
20	31	Moderate
30	43	Moderate
40	51	High

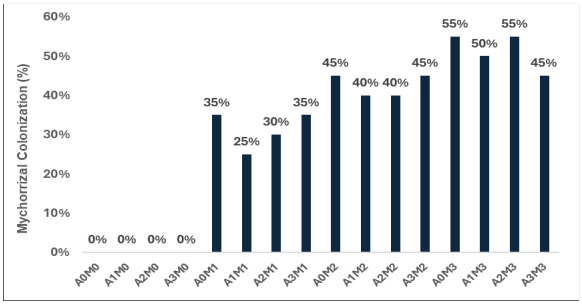

Fig. 15: Arbuscular mycorrhizal colonization (%) in the roots of citronella (*Cymbopogon nardus*) under different salicylic acid (SA) and arbuscular mycorrhizal fungi (AMF) treatments.

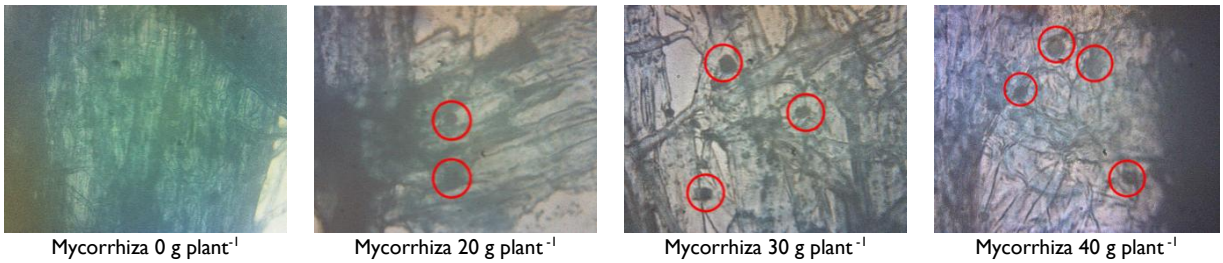

Fig. 16: Vesicles in the roots of citronella grass (*C. nardus*).

4. DISCUSSION

The present study showed that the response of vegetative growth to SA and AMF varied among the measured traits. For leaf number, a significant SA × AMF interaction was observed, indicating that the response to SA depended on the level of AMF inoculation. The highest leaf number was observed at 200 mg L^{-1} SA combined with either 0 or 20 g plant^{-1} AMF, whereas the lowest value was recorded at 0 mg L^{-1} SA combined with 20 g plant^{-1} AMF. In contrast, tiller production was not significantly affected by SA, AMF, or their interaction. Although numerical variation in tiller number was observed across the tested SA concentrations and AMF inoculation levels, these differences did not reach statistical significance. Therefore, the observed variation in tiller production should be interpreted as a numerical response rather than evidence of a treatment effect or a combined SA–AMF response. The contrasting responses of leaf and tiller production indicate that the effects of SA and AMF on vegetative development were trait-specific under the experimental conditions. The significant interaction observed for leaf production may be associated with the complementary physiological roles of SA and AMF. SA has been reported to enhance ion uptake, stabilize cellular membranes, improve photosynthetic performance, promote proline accumulation, and activate antioxidant defense systems, which collectively contribute to plant adaptation to stress (Roshdy et al., 2021; Thepbandit et al., 2024; Ilyas et al., 2024). Likewise, AMF can facilitate plant growth by improving nutrient and water acquisition, enhancing phosphorus mobilization through phosphatase activity, and increasing the effective absorptive surface area of roots through extraradical hyphae (Diagne et al., 2020; Danata et al., 2025). These complementary functions may contribute to the interaction observed for leaf production. However, the absence of a significant SA × AMF interaction for tiller production indicates that this response was less responsive to the combined treatments under the conditions evaluated.

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Neither salicylic acid (SA), arbuscular mycorrhizal fungi (AMF), nor their interaction significantly affected chlorophyll b or total chlorophyll content. Nevertheless, a numerical variation among the treatment combinations was observed, with the highest mean chlorophyll values recorded at the higher SA concentrations. Application of 150–200 mg L^{-1} SA resulted in numerical increases in chlorophyll b and total chlorophyll of up to 52.17 and 20.97%, respectively, relative to the control, although these differences were not statistically significant. Such trends are consistent with previous reports indicating that SA may contribute to the maintenance of chlorophyll accumulation by preserving chloroplast integrity and protecting the photosynthetic apparatus from oxidative damage (Choudhary et al., 2021; Omidi et al., 2022). AMF inoculation likewise did not significantly affect chlorophyll b or total chlorophyll content. This result suggests that the increase in biomass observed under AMF inoculation was not accompanied by a detectable change in photosynthetic pigment concentration under the conditions evaluated. Nevertheless, AMF significantly enhanced shoot biomass, with shoot fresh and dry weights increasing by up to 71.93% and 65.55%, respectively, compared with the control. This response is consistent with previous findings that AMF can promote plant growth through improved acquisition of water and mineral nutrients, particularly phosphorus, facilitated by the extensive extraradical hyphal network associated with colonized roots (Wahab et al., 2023; Xiao et al., 2023b; Boyno et al., 2025). The contrasting responses of chlorophyll concentration and biomass indicate that the positive effect of AMF on plant biomass in this study was not necessarily mediated by changes in chlorophyll accumulation. The absence of a significant SA and AMF interaction for chlorophyll b and total chlorophyll further indicates that the combined application of the two treatments did not produce a detectable interactive effect on photosynthetic pigment accumulation. Thus, unlike leaf and tiller production, for which a significant SA and AMF interaction was observed, chlorophyll accumulation appeared relatively stable across the treatment combinations. The lack of a significant response of chlorophyll content to SA may also reflect the concentration range, timing of application, developmental stage, or environmental conditions under which the experiment was conducted. Previous studies have reported that the effects of exogenous SA on chlorophyll and other physiological traits can vary according to application concentration, plant genotype, developmental stage, and growing conditions (Wassie et al., 2020; Rehman et al., 2022). Similarly, the effects of AMF on photosynthetic pigments are not necessarily consistent across plant species and experimental conditions, even when AMF colonization results in improvements in plant growth and biomass. Therefore, the present results suggest that SA and AMF affected different aspects of citronella plant performance, with the clearest responses observed in vegetative growth and biomass rather than in chlorophyll b or total chlorophyll accumulation.

The response of essential oil production revealed distinct numerical patterns in relation to SA application and mycorrhizal inoculation. The highest essential oil yield was recorded in the combination of 200 mg L^{-1} SA and 30 g $plant^{-1}$ mycorrhiza, reaching 0.105 g $plant^{-1}$ (4.2 kg ha^{-1}), while mycorrhizal inoculation at 40 g $plant^{-1}$ without SA produced a relatively high yield of 0.082 g $plant^{-1}$ (3.28 kg ha^{-1}). These observations indicate that mycorrhizal inoculation was associated with greater essential oil production, potentially in relation to the greater biomass accumulation observed under AMF treatments and their capacity to improve plant nutrient acquisition (Golubkina et al., 2020; Begum et al., 2021). In contrast, the highest essential oil content (0.072%) was observed under 100 mg L^{-1} SA without mycorrhizal inoculation. This pattern differed from that observed for essential oil yield, indicating that the treatment associated with the highest oil content was not the same as that associated with the highest oil yield. Similar variation in essential oil responses to SA application has been reported in aromatic plants, where SA can alter essential oil accumulation and composition depending on concentration and growing conditions (Ghassemi-Golezani & Farhadi, 2022). The combination of 200 mg L^{-1} SA and 40 g $plant^{-1}$ mycorrhiza, despite being associated with high biomass production, showed the lowest essential oil content (0.027%). This inverse pattern may suggest a potential biomass dilution effect; however, this mechanism was not directly evaluated in the present study and therefore remains a hypothesis.

Gas chromatography–mass spectrometry (GC–MS) analysis revealed differences in the relative composition of essential oil constituents between the control (A0M0) and the selected combined treatment (A2M3; 200 mg L^{-1} SA + 30 g $plant^{-1}$ mycorrhiza). Citronellal, citronellol, and geraniol were the predominant constituents in both treatments, although their relative proportions differed between the two profiles. In the selected combined treatment, citronellal accounted for 19.87% of the total relative peak area, compared with 15.28% in the control, whereas citronellol and geraniol accounted for 16.73% and 41.32%, respectively, compared with 19.27% and 44.80% in the control. These differences indicate that the selected combined treatment was associated with a distinct relative profile of the major essential oil constituents compared with the control. Several minor constituents, including α-pinene, citronellyl acetate, geranic acid, and trans-caryophyllene, also showed differences in their relative proportions between the two treatments. Such variation in essential oil composition is consistent with previous studies reporting changes in essential oil profiles following the application of elicitors or mycorrhizal inoculation in aromatic plants, including peppermint and basil (Youssef 2021; Afkar, 2024).

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The relative proportions of the major constituents in the selected essential oil profile were higher than the compositional ranges specified in ISO 3849:2003 for geraniol (3.0–8.5%), citronellol (4.0–7.0%), and citronellal (7.0–11.5%). The observed deviations in constituent proportions may reflect differences in cultivar, environmental conditions, plant maturity, cultivation practices, and essential oil extraction procedures (Abduh et al., 2020; Tajjudin et al., 2024; Salima et al., 2025). In addition to pre-harvest factors, post-harvest refinement techniques such as vacuum fractional distillation have been reported to modify the relative proportions of citronellal and geraniol, thereby improving the compositional characteristics of citronella oil (Le et al., 2023; Ahmad et al., 2025). Mycorrhizal colonization analysis provided additional insight into the establishment of AMF symbiosis in citronella grass. No mycorrhizal colonization was detected in treatments without AMF inoculation, whereas inoculated plants exhibited varying levels of root colonization. Based on the observed responses across AMF doses, colonization increased from 31% at 20 g plant^{-1} to 43% at 30 g plant^{-1} and 51% at 40 g plant^{-1}. At the individual treatment-combination level, the highest colonization (55%) was observed in the A2M3 treatment (150 mg L^{-1} SA + 40 g plant^{-1} AMF), while the A0M3 treatment (0 mg L^{-1} SA + 40 g plant^{-1} AMF) also showed 55% colonization. Colonization under A1M3 and A3M3 reached 50% and 45%, respectively. These patterns indicate that AMF inoculation was associated with the establishment of root colonization, while variation among SA concentrations suggests that the response of AMF colonization may depend on the SA level. The relatively high colonization observed under some SA and AMF combinations may be related to the influence of SA on plant–microbe interactions and root physiological conditions. SA is involved in plant defense signaling and may influence the establishment and development of symbiotic associations, although its effects can vary depending on concentration, plant species, and fungal symbiont (Benjamin et al., 2022; Hou & Tsuda, 2022). In the present study, the highest colonization was observed at 40 g plant^{-1} AMF, particularly in the absence of SA and at 150 mg L^{-1} SA, whereas the 200 mg L^{-1} SA treatment showed a lower colonization level. This pattern suggests a possible concentration-dependent response of AMF colonization to SA. The results indicate that AMF inoculation was important for establishing root colonization in citronella grass, while variation in SA concentration was associated with differences in the extent of colonization.

5. CONCLUSION

The present study demonstrates that salicylic acid (SA) and arbuscular mycorrhizal fungi (AMF) influenced different aspects of citronella grass (*Cymbopogon nardus* L.) performance, with responses varying among plant traits. AMF inoculation showed a consistent positive contribution to shoot biomass, significantly increasing shoot fresh and dry weights, whereas the SA × AMF interaction was particularly evident in leaf production. These findings indicate that AMF can play an important role in supporting vegetative biomass development, while the response of specific growth traits to SA may depend on AMF inoculation. Essential oil production also showed promising treatment-dependent variation, with the highest observed oil yield obtained at 200 mg L^{-1} SA combined with 30 g plant^{-1} AMF, while the highest oil content was observed at 100 mg L^{-1} SA without AMF. Although these responses were descriptive and do not support designation of a single optimal treatment, they demonstrate the potential of SA and AMF treatments to influence different components of essential oil production. GC–MS profiling further revealed differences in the relative proportions of the major essential oil constituents between the untreated control and the selected combined treatment, indicating variation in oil quality characteristics associated with treatment. Overall, the findings highlight AMF as a promising input for improving citronella biomass production and suggest that appropriate SA–AMF combinations may be useful for modulating specific growth and essential oil traits. Further field validation across cultivars, environments, and successive harvests is warranted to establish the consistency and agronomic applicability of these responses.

Declarations

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Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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