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Effect of Magnesium Sulfate and Rhizosphere Microorganisms on the Growth and Biosynthesis of *Cymbopogon nardus* L. Essential Oil

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ABSTRACT

Background: Citronella oil from *Cymbopogon nardus* L. is used in various fields, including health, industry and pharmaceuticals. Efforts to increase the content and quality of essential oils in this research were made with the application of magnesium sulfate. Rhizosphere microorganisms, which act as biological fertilizers, are applied to assist in the absorption of nutrients. This research aims to determine the interaction between the type of rhizosphere microorganisms and the dose of $MgSO_4$ on growth, essential oil yield and essential oil content in *C. nardus*.

Methods: The research was carried out from February to August 2023. The research used a randomized block design arranged factorially with three replications. The first factor is the type of rhizosphere microorganisms (without RM, PGPR and AMF) and the second factor is the $MgSO_4$ fertilizer dose (0, 190, 285 and 280 kg ha^{-1}).

Result: Providing $MgSO_4$ fertilizer can increase the growth and yield of essential oils of *C. nardus*. The application of PGPR and AMF can increase efficiency of $MgSO_4$ fertilization. Treatment without administering rhizosphere microorganisms required a higher dose of $MgSO_4$ compared to PGPR and AMF treatments. To increase growth variables and essential oil quality, treatment without rhizosphere microorganisms requires $MgSO_4$ of 380 kg ha^{-1}, while PGPR and AMF only require $MgSO_4$ at a dose of 190-285 kg ha^{-1}.

Key words: AMF, *Cymbopogon*, Essential oils, $MgSO_4$ fertilizer, PGPR.

INTRODUCTION

C. nardus, as a producer of citronella oil, is in demand by various countries include the United States with a demand of 698.8 tons per year, France with 302.3 tons per year, England 205.75 tons per year, the Netherlands with 86.5 tons per year and Germany with 77.5 tons (Camacho *et al*., 2015). Citronella oil is used as a raw material for making soap, perfume, cosmetics, insect repellent products (especially mosquitoes), body care to the pharmaceutical sector for health (Hamzah *et al*., 2014; Zulfikar and Sitepu, 2019; Kaur *et al*., 2021). The main components contained in citronella oil include Citronellal, Citrinellol, geraniol, camphene, limonene, 1-borneol, methyl isoeugenol and geranyl formate. The main ingredients found in large quantities include citronellal (16-36%), citronellol (4-13%) and geraniol (7-22%), then minor components include eugenol (0.78-2.5%), δ-cadinene (0.36-1.09%), β-myrcene (0.09-2.9%) (Heiba and Rizk, 1986; Kaur *et al*., 2021).

The chemical compounds in essential oils are influenced by geographical conditions, environmental factors, ecological conditions, climate, plant growth phase, harvest time, genetic factors and extraction methods (Kaur *et al*., 2021). Efforts that can be made to increase the tolerance of *C. nardus* in shade conditions include management and nutrient uptake in the soil, it can be done through fertilization. Essential nutrients needed by plants include magnesium and sulfur. Magnesium is part of chlorophyll, precisely as the core of chlorophyll, so it has a vital role in photosynthesis. Apart from that, magnesium also has a role in the active component of several enzymes, in oxidative phosphorylation to form ATP, in the citric acid cycle and production of oil in plants (Soetan *et al*., 2010; Nejatzadeh, 2020). Sulfur is an essential component in primary and secondary metabolic processes. In plants, small sulfur-containing biomolecules are required to maintain cellular homeostasis, including respiration, photosynthesis and various secondary metabolisms (Nakai and Maruyama-Nakashita, 2020). Giving sulfur to *Cymbopogon martini* (Roxb.) palmarosa plants at the highest dose, namely 40 kg ha^{-1}, produces the highest biomass and total essential oil yield (Rajeswara Rao *et al*., 2015).

A good soil ecosystem can support plant root growth by improving the physical, biological and chemical properties of the soil.Rhizosphere microorganisms are microorganisms that have many benefits for plant growth. These microorganisms can be bacteria better known as

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PGPR (plant growth promoting rhizobacteria) as well as fungi known as mycorrhiza or AMF (arbuscular mycorrhizal fungus). PGPR is able to act as biofertilizers, biostimulants and biocontrol by increasing the supply and availability of nutrients, producing phytohormones and increasing plant resistance from pest attacks (Odoh, 2017). AMF is a fungus that has a symbiotic relationship with plant roots that forms vesicles, arbuscules and hyphae in root cells to increase growth, water and nutrient absorption and increase stress tolerance (Songachan *et al*., 2023). Apart from their role in supporting plant growth, PGPR and AMF also increase secondary metabolites such as essential oils. Inoculation of AMF *Glomus fasciculatum* or *Glomus mosseae* on basil plants showed a fourfold increase in essential oil accumulation compared to those without inoculation (Zolfaghari *et al*., 2012). This research aims to study the effect of Mg and S fertilization and inoculation of PGPR and AMF on the growth and essential oils of *C. nardus*.

MATERIALS AND METHODS

Experimental detail

C. nardus field experiment was conducted at Jatimulyo Village, Lowokwaru Sub-district, Malang, East Java, Indonesia, during February-August 2023. The research was carried out with a factorial randomized block design consisting of two factors. The first factor, rhizosphere microorganisms consists of three levels, *i.e.*, without rhisozphere microorganisms (without RM); PGPR and AMF. The second factor, dose of $MgSO_4$ consists of four levels, *i.e.*, 0 kg ha^{-1}; 190 kg ha^{-1} , 285 kg ha^{-1}, 380 kg ha^{-1}. There were 12 combinations of treatments and 3 three times repetitions, resulting in 36 experimental units.

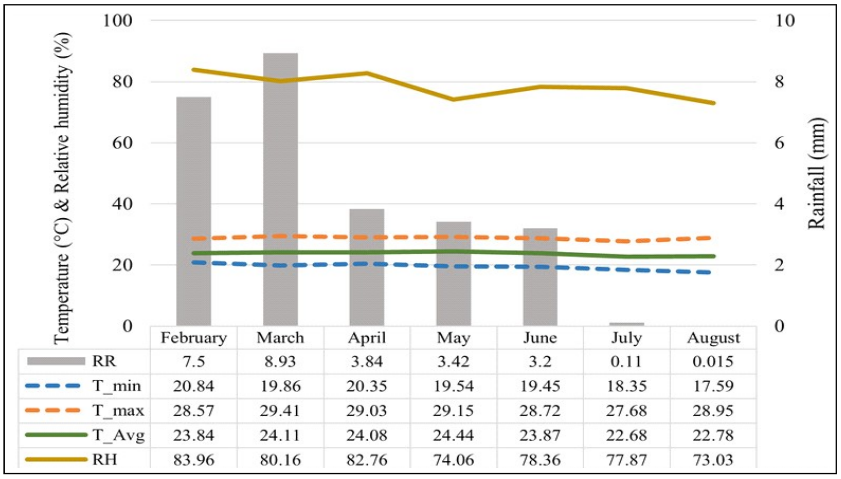
Field experiment

Field experiments were carried out on dry land with pH 7.27, C-Organic 1.37%, total N 0.10%, organic matter 2.36%, P_2O_5 9.18 ppm, K 0.16 me, Mg 1.41 $cmol^+/kg$ and SO_4 19.82 ppm. The experimental site is situated at an altitude of approximately 445 meters above sea level (masl), average air temperature 22.5-24.5°C, average relative humidity 73-84% and average rainfall 0.015-8.93 mm $month^{-1}$ (Fig 1). The citronella planting material used is vegetative propagation taken from Seraiwangi 1 varieties that are > 1 year old. The planting distance used is 50×50 cm planted under 25% shading treatment. The PGPR used contains *Bacillus* sp., *Pseudomonnas* sp., *Azotobacter* sp. and Azospirillum sp. while the mycorrhiza used is included in the endomycorrhiza/AMF originating from the Glomus sp isolate. PGPR application was given at a concentration of 10 ml L^{-1} with a dose of 20 mL $plant^{-1}$ which is given by pouring it on the plant roots at the time of transplanting, 45, 90 and 135 days after planting (Hamed *et al*., 2017; Larasati *et al*. (2022); Komansilan *et al*., 2023). The application of AMF granules is given at the time of transplanting at a rate of 50 g $plant^{-1}$ which is applied to the planting hole (Kumar *et al*., 2021). $MgSO_4$ fertilizer is given according to the treatment levels.

Measurement

The growth variables measured included the number of leaves, leaf area, fresh weight and dry weight of *C. nardus*. Leaf area is measured with a leaf area meter (LAM) using the Average Leaf Area (ALA) method (Widaryanto *et al*., 2019). Dry weight observations were carried out by drying the samples in an oven at 80°C for 24 hours and then weighing them. To obtain the yield (Essential oil content), distillation is carried out using steam distillation for approximately 4 hours starting from the first steam droplet. The oil content is calculated using the equation:

$$\text{Essential oil content} = \frac{\text{Volume of essential oil collected (ml)}}{\text{Weight of fresh herb (g)}} \times 100\%$$



	February	March	April	May	June	July	August
RR	7.5	8.93	3.84	3.42	3.2	0.11	0.015
T_min	20.84	19.86	20.35	19.54	19.45	18.35	17.59
T_max	28.57	29.41	29.03	29.15	28.72	27.68	28.95
T_Avg	23.84	24.11	24.08	24.44	23.87	22.68	22.78
RH	83.96	80.16	82.76	74.06	78.36	77.87	73.03

Fig 1: Monthly mean climatological data (temperature, rainfall and relative humidity).

Essential oil yield was calculated based on the weight of fresh herbs using formula:

Essential oil yield =

$$\frac{\text{Weight of fresh herbs (g)}}{100} \times \text{Oil content}$$

To determine the chemical compounds contained in citronella essential oil, analysis was carried out using GC-MS (Gas Chromatography-Mass Spectrometry) QP-2010S/ Shimadzu. The collected data were analyzed using analysis of variance and then examined with the F test at a 5% error level and further with duncan multiple range test (DMRT) at a 5% error level.

RESULTS AND DISCUSSION

Plant growth

Using rhizosphere microorganisms and $MgSO_4$ fertilizer doses has been proven to increase growth variables, including the number of leaves, leaf area, fresh weight and dry weight of plants. Observing the number of leaves and the leaf area, without RM treatment continued to increase up to a $MgSO_4$ dose of 380 kg ha^{-1}, in the PGPR treatment increased when 285 kg ha^{-1} was given, while the AMF treatment increased at a $MgSO_4$ dose of 190-285 kg ha^{-1} (Fig 2 and 3). When observing fresh weight and dry weight, without RM treatment required a $MgSO_4$ dose of up to 380 kg ha^{-1}, while the PGPR and AMF treatments increased at a $MgSO_4$ dose of 190-285 kg ha^{-1} and decreased when giving $MgSO_4$ 380 kg ha^{-1} (Fig 4 and 5).

Increased growth of *C. nardus* is influenced by the adequacy of the essential nutrients Mg and S through $MgSO_4$ fertilization. Mg has a role in photophosphorylation (formation of ATP in chloroplasts), activation of the RuBP enzyme, CO_2 fixation, protein synthesis, chlorophyll formation and transport in the phloem as a buffer for photosynthesis (Cakmak and Kirkby, 2008; Kanjana, 2020). S has a role that is no less important; S is a constituent of protein amino acids such as methionine, cysteine, glutathione, vitamins (biotin and thiamine), phytochelatin,

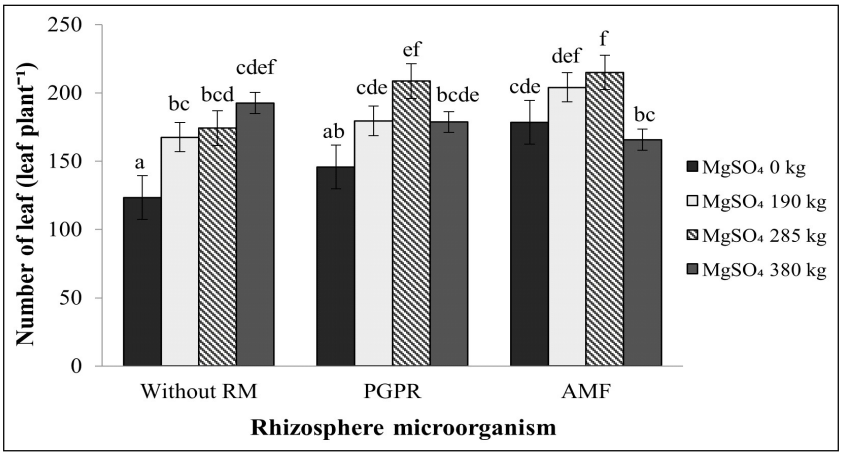


Fig 2: Interactions between rhizosphere microorganisms and $MgSO_4$ on the number of leaf of *C. nardus*.

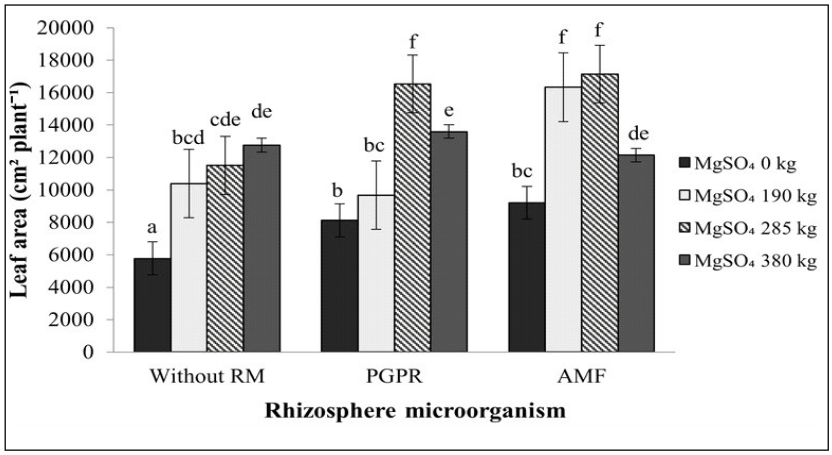


Fig 3: Interactions between rhizosphere microorganisms and $MgSO_4$ on leaf area of *C. nardus*.

chlorophyll and coenzyme A (Narayan *et al*., 2022). Increasing the content of protein, vitamins, starch, carbohydrates, glycation and chlorophyll due to the administration of Mg and S increases leaf area, plant height, fresh weight and plant dry weight (Riffat and Ahmad, 2020). Mg fertilization has an influence on the growth of microorganisms such as nitrogen-fixing bacterias and AMF (Soniya and Bhindhu, 2023). Treatment without applying rhizosphere microorganisms requires more $MgSO_4$ fertilizer than with the addition of PGPR or AMF. Rhizobacteria are able to help absorb S, which is not available in the soil. Sulfate-ester mineralization is catalyzed by sulfatase from the esterase class, with the enzymes involved being arylsulfatase and alkylsulfatase. These two enzymes break down the O-S bonds, which then release sulfate in the rhizosphere (Kertesz, 1999; Kertesz and Mirleau, 2004).

Based on research that has been carried out, increasing the dose of $MgSO_4$ fertilizer can increase the chlorophyll content of *C. nardus* (Fig 6). Mg and S are two essential elements in the formation of chlorophyll. Mg is the only mineral in the central atom of chlorophyll, so plants that experience Mg deficiency will experience interveinal chlorosis, namely the degradation of chlorophyll between the bones of plant leaves (Kanjana, 2020). Apart from Mg, S deficiency in plants causes the chlorophyll and Rubisco content to decrease, thereby inducing chlorophyll in young leaves (Chowdhury *et al*., 2020). Apart from Mg and S fertilization, the increase in chlorophyll is also influenced by the application of rhizosphere microorganisms. PGPR inoculation (*Pantoea agglomerans* and *Bacillus* sp.) and AM (*Rhizophagus fasciculatus* and *Rhizophagus aggregatum*) able to increase total chlorophyll in plants even under stress conditions such as salinity (Diagne *et al*., 2020).

Yield and quality of essential oils

The use of the PGPR can increase the essential oil content by 2.32%, while the use of AMF can increase it by 5.14% compared to without RM (Fig 7). $MgSO_4$ fertilization with doses of 190 kg ha^{-1} and 285 kg ha^{-1} can increase essential oil content by 6% and a dose of 380 kg ha^{-1} only increases 0.54%. When observing the weight of fresh herbs and essential oil yield, without RM treatment required a dose of $MgSO_4$ 380 kg ha^{-1}, PGPR 285 kg ha^{-1} and AMF 190-285 kg ha^{-1} (Fig 8 and 9). Giving sulfur to *Cymbopogon martini* (Roxb.)

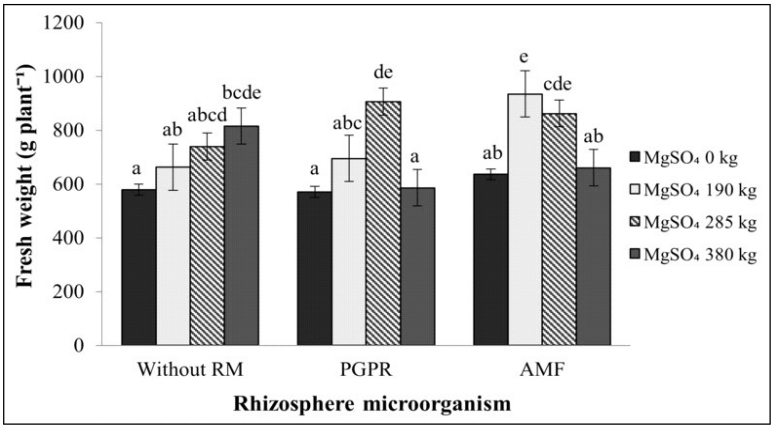


Fig 4: Interactions between rhizosphere microorganisms and $MgSO_4$ on fresh weight of *C. nardus*.

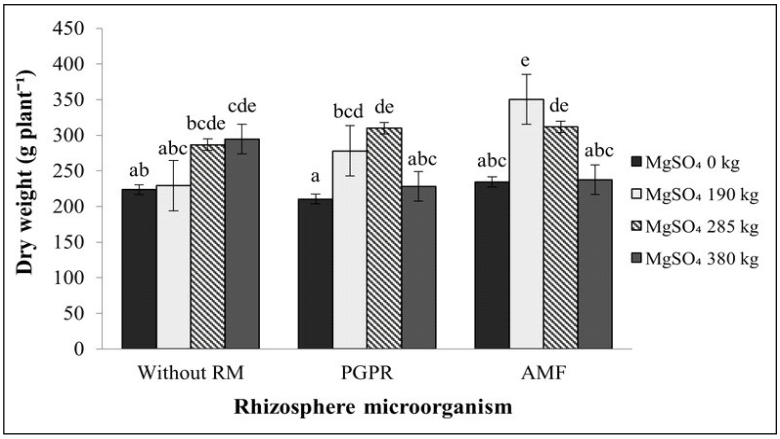


Fig 5: Interactions between rhizosphere microorganisms and $MgSO_4$ on the dry weight of *C. nardus*.

palmarosa plants at the highest dose, namely 40 kg ha^{-1}, produces the highest biomass and total essential oil yield. This is because sulfur is an important component in the preparation of acetyl Co-A, which acts as a terpene precursor (Rajeswara Rao *et al*., 2015). The application of PGPR and AMF can increase the efficiency of $MgSO_4$ fertilization. The hyphae of AMF allow bacterial colonization, both of which are able to provide nutrients. PGPR and Rhizobium inoculation is proven to minimize the occurrence of plant nutrient deficiencies and can increase soil fertility, productivity and profitability (Neha *et al.*, 2024). AMF can increase plant accessibility to obtain N,P,K and S elements in the soil through hyphae growth to expand root uptake (Bisht and Garg, 2022). The abundance of desulfonating bacteria due to increased AMF root colonization allows S uptake for plants (Gahan and Schmalenberger, 2014). Inoculation of *Septoglomus viscosum* (syn. *Glomus viscosum*) can increase the yield of essential oils in oregano plants (Tarraf *et al*., 2015).

The main components of citronella essential oil are citronellal, citronellol and geraniol (Fig 10). The compound with the highest percentage found was citronellal, ranging from 36.00-43.64%, followed by geraniol with a percentage of 20.56-22.66% and finally citonellol 14.95-16.56%. Previous research shows that applying sulfur can increase the yield of essential oils and the composition of essential oils in *Ocimum basilicim* L. (Oliveira *et al*., 2014). Essential oils are terpenoids (monoterpenes) which come from the synthesis of acetyl-coenzyme A (Gusmaini and Syakir, 2020). Terpenoids are synthesized from the same precursor, namely isopentenyl pyrophosphate (IPP) and its isomer dimethyl-lallyl pyrophosphate (DMAPP). The biosynthesis of IPP and DMAPP in plants is carried out through two biosynthetic pathways, namely the MVA (mevalonic acid) and MEP (2-C-methylerythritol-4-phosphate) pathways. Most secondary metabolites come from amino acids or acetyl-coenzyme A, the MVA pathway starts from the condensation of three acetyl-Co-A molecules, which then form mevalonic acid (Ahmed *et al*., 2017). Acetyl Co-A is an important molecule in plant metabolism that contains the element S with the chemical formula $C_{23}H_{38}N_7O_{17}P_3S$. Supplementation of sulfur (S) can increase the concentration of acetyl Co-A and acetyl Co-A carboxylase activity (Ahmad *et al*., 2000). S fertilization combined with N fertilizer at different locations had a significant effect on essential oil yields (Zheljazkov *et al*., 2008).

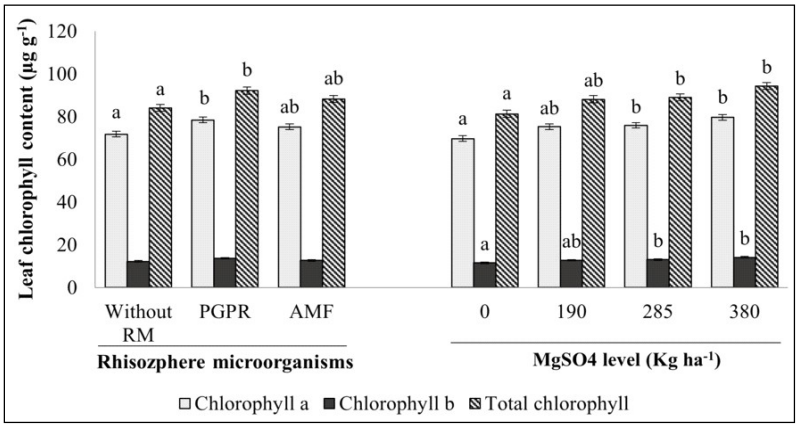


Fig 6: Effect of different rhizosphere microorganisms and $MgSO_4$ levels on leaf chlorophyll content of *C. nardus.*

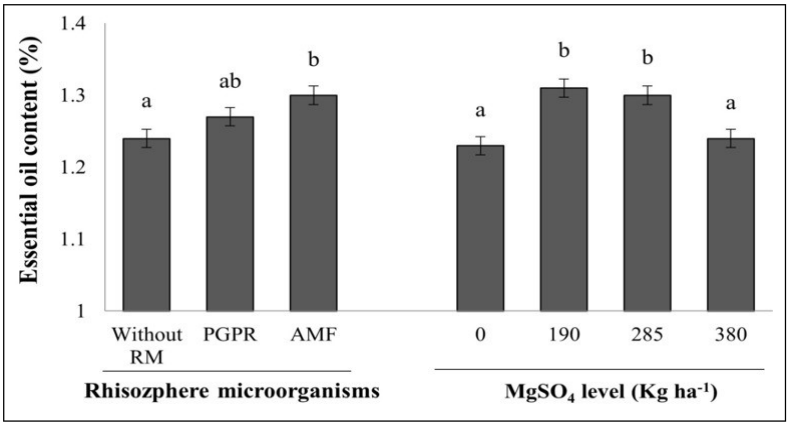


Fig 7: Effect of different rhizosphere microorganisms and $MgSO_4$ levels on the essential oil content of *C. nardus.*

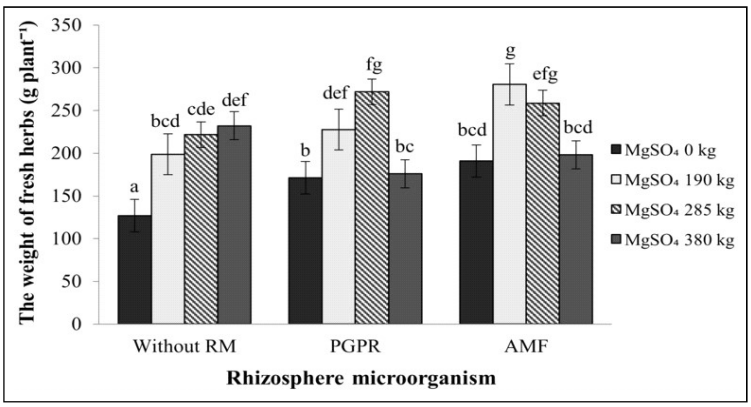


Fig 8: Interactions between rhizosphere microorganisms and $MgSO_4$ on the weight of fresh herbs of *C. nardus*.

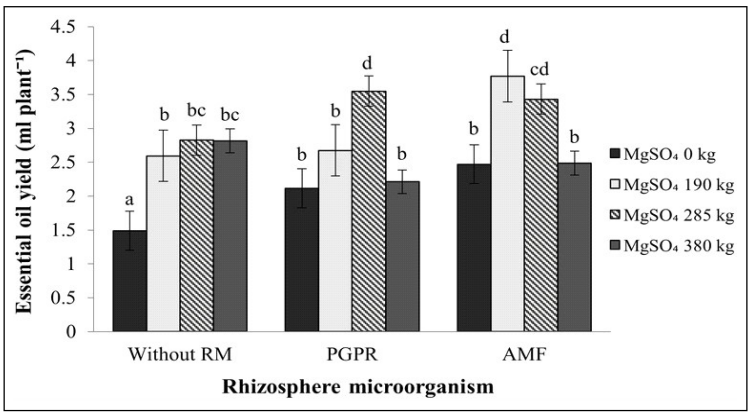


Fig 9: Interactions between rhizosphere microorganisms and $MgSO_4$ on essential oil yield of *C. nardus*.

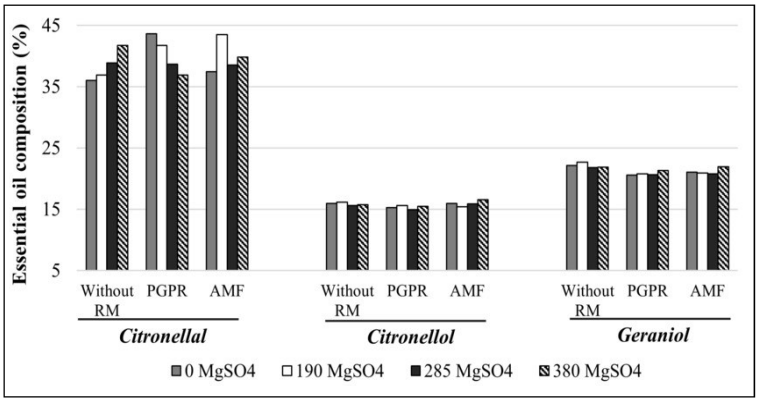


Fig 10: Effect of different rhizosphere microorganisms and $MgSO_4$ levels on essential oil composition of *C. nardus*.

CONCLUSION

PGPR and AMF can increase the growth variables and essential oils of *C. nardus* compared to without rhizosphere microorganisms. $MgSO_4$ fertilization can increase the growth and essential oils of *C. nardus* at certain doses. In treatment without the application of rhizosphere microorganisms 380 kg ha^{-1} of $MgSO_4$ is required, while the use of PGPR and AMF only requires $MgSO_4$ at a dose of 190-285 kg ha^{-1}. The use of PGPR and AM can increase $MgSO_4$ fertilization efficiency by 49% to 53% compared to without rhizosphere microorganisms.

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Conflict of interest

There is no conflict of interest.

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