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# Volatile Organic Compounds (VOCs) Emission and Antioxidant Activities of Rice Treated with a Consortium of Plant Growth-Promoting Rhizobacteria under Drought Conditions

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**Abstract** Plants are known to release various volatile compounds under stress conditions. When inoculated on crops, plant-growth promoting Rhizobacteria (PGPR) produces volatile organic compounds (VOCs), thus enhancing crop seedling production, crop weight, crop yield, and stress resistance. In this study, non-inoculated and inoculated rice plants with a PGPR consortium were set up in glasshouses under drought and non-stress conditions. Drought stress was applied for six days, after which water was added to maintain plant growth. Under both conditions, 68 VOCs were found in rice leaves. The volatile organic compounds (VOCs) were quantified using the solid-phase microextraction technique paired with gas chromatography mass spectrometry (SPME-GCMS). Ethylene oxide (EO) was detected in drought-stressed plants compared to that of ethylene. The percentage of EO in non-inoculated rice was higher relative to inoculated rice plants under drought conditions. The identified VOCs in the inoculated and non-inoculated rice (drought stress and non-stress) belonged to the chemical classes of aldehyde, alcohol, terpene, ketone, ester, ether, amine esters, amides, and others. The enzymatic antioxidant activity of rice leaves was also determined to scavenge reactive oxygen species (ROS). Superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (GPX) levels significantly increased in their activities in inoculated rice compared to non-inoculated under drought stress. Our research focused on how drought affects plant metabolism above and below ground to adapt to a stressful environment.

**Keywords** PGPR, Drought-Tolerant, Vocs, Enzymatic Antioxidant, Inoculant

## 1. Introduction

Abiotic stress causes plants to send signals to their metabolite systems, thus responding to the stress level. For crops under stress conditions, generating reactive oxygen species (ROS) signals an antioxidant activity response.

Plants produce VOCs for stressed crops. Plants use transpiration to transfer volatile compounds from roots to leaves, flowers, and fruits. The profile of VOCs emitted by microorganisms in the field is determined by soil properties, the microbial community, plant exudates, and internal factors that influence the metabolism of each microbial strain [1].

Soil heterogeneity influences the bacterial VOC spectrum, which metabolites released by bacteria or plants modify. Some of the VOCs produced by bacteria are species-specific and are used to classify microorganisms [1]. The most common sources of volatile chemicals are metabolic byproducts of anaerobic fermentation processes and extracellular breakdown of complex organic molecules. According to Wenke et al. [2], bacterial scents serve as signalling chemicals for interspecies and intraspecies communication or cell-to-cell communication, eliminating excess carbon compounds or as stimulants or inhibitors of growth. PGPR strains *B. amyloliquefaciens* IN937 and *B. subtilis* GB03 inoculated in *Arabidopsis* seedlings have been discovered to produce acetoin (3-hydroxy-2-butanone), a molecule that promotes development and resistance [3]. *Pseudomonas sp.* and *Bacillus sp.* have produced 1-Heksanol as a plant growth promoter and an antifungal compound [4].

Volatile organic compounds (VOCs) can undergo adsorption, desorption, or reaction with clay surfaces, allowing them to diffuse through the soil, water, or air in the rhizosphere [5, 6]. SPME has been widely used for VOC analysis [7]. The benefits of headspace SPME method include its simplicity, ability to reuse samples, high sensitivity, and repeatability [8]. The type of fibre used in SPME absorbs compounds based on their polarity and size. These fibres absorb low polar molecular weight VOCs.

ROS damages the cell membrane's structure by oxidising polyunsaturated fatty acids of the lipid layer and altering its permeability. However, plants have an excellent antioxidant system to improve oxidative stress caused by ROS by producing enzymatic and non-enzymatic antioxidants [9]. Several studies have shown that enzymatic antioxidants such as ascorbate peroxidase (APX), CAT, and SOD, as well as non-enzymatic antioxidants like carotenoids, proline, -tocopherols, and ascorbic acid, can help to fight oxidative stress [9].

Limited research has been carried out on VOCs produced by rice crops inoculated with mixed PGPR inoculants under drought stress and analysis on the effect of enzymatic antioxidants. This article discussed some VOCs emitted by inoculated rice under stress and plant defence mechanisms against ROS. It is hoped that the findings from this present study on the production of VOCs by plants could serve as a guide for determining the status of plants immediately, whether they are stressed or not, since different plant species emit different types of VOCs. In contrast, analysing enzymatic antioxidant activity served as a quantitative measurement of plant stress levels. This study aimed to determine (1) the types of volatiles emitted and (2) the antioxidant activities in treated rice with and without PGPR consortium under drought conditions.

# 2. Materials and Methods

### 2.1. Rice Plant Preparation and Plant Sampling

Seeds of the MR219 rice variety were obtained from the Malaysian Agricultural Research and Development Institute (MARDI) Genebank. The PGPR consortium consisted of *Bacillus altitudinis* UKM RB11, *Bacillus cereus* UKM R66, and *Achromobacter spanius* UR10, which were isolated from rice rhizospheres, and was used in this study. A proportion of $OD_{600nm}$ 1.0 bacterial cell suspension was used for the inoculation treatment (T3, T4). Rice seeds (variety MR219) were surface-sterilised for 15 minutes with a 3 % bleach solution and then washed three times with sterilised distilled water. The inoculant suspension was applied to the dried seeds and incubated for 24 hours at 28 ℃. The inoculated seeds were germinated for up to 6 days, then transplanted into pots filled with sterilised clay soil. All rice plants were irrigated daily except for the treatment for drought stress initiated at the flowering stage, which involves withholding water for 5 days for inoculated and non-inoculated rice. The leaves were collected for further analysis at the end of the drought treatment, and the rice plants were watered to maintain growth until maturity.

### 2.2. Solid Phase Micro Extraction-Gas Chromatogram-Mass Spectrophotometry (SPME-GCMS) Analysis

The rice leaves samples were collected directly from the glasshouse early in the morning. Approximately 10 cm of leaves were cut into 2cm to 3cm long and immediately transferred into a 20ml headspace vial with septum cover. SPME was performed using 75 µm Carboxen/Polydimethylsiloxane fibre (Sigma, USA). This analysis was conducted at the Center for Research Management and Instrumentation (CRIM), Universiti Kebangsaan Malaysia.

### 2.3. Quantitative Determination of Enzymes

Leaf tissue weighing 100 mg was subjected to cryogenic pulverisation and subsequent homogenisation. A sample of 1g of fresh leaves was washed with sterilised distilled water and macerated with 5 ml phosphate buffer (pH 7.8) in an ice-cooled pestle-mortar at 4 ℃. The extract was centrifuged at 15,000rpm for 15 minutes at 4 ℃ for enzymatic tests.

#### 2.3.1. Superoxide Dismutase (SOD)

The method to measure SOD activity is described by Marklund and Marklund [10]. Approximately 3ml of the reaction mixture containing Tris-HCL buffer and 10mM of EDTA is used. For this reaction mixture, 100 µl of enzyme extract must be rapidly added, mixed well, and incubated at 25 ℃ for 10 minutes. The reaction was stopped by adding 50µl of 10mM HCl, and the absorbance rate was measured at 420 nm.

#### 2.3.2. Catalase (CAT)

The Rao et al. [11] method was used to measure catalase activity [11]. A reaction mixture consisting of 3 ml of phosphate buffer (300 µM, pH 7.2) and $H_2O_2$ (100 µM) in enzyme extract (1 ml) was allowed to release $O_2$ by enzymatic dissociation of $H_2O_2$ in the dark for 1 minute. $O_2$ produced due to enzyme reaction was determined at $OD_{240nm}$ (extinction coefficient of $H_2O_2$ is 0.036 $mM^{-1}$ $cm^{-1}$). The enzyme's activity is expressed as µM $H_2O_2$ oxidised U $g^{-1}$ protein.

#### 2.3.3. Guaiacol Peroxidase (GPX)

The GPX enzyme was estimated based on the method of Zhang et al. [12]. Approximately 1 ml of 40 mM guaiacol (v/v), 1 ml of 10 mM $H_2O_2$ (v/v) and 4 ml of 100 mM of potassium phosphate buffer (pH 6.5) were mixed into 6 ml of the mixed solution. Afterwards, 10 µl of enzyme extract was added immediately to initiate the reaction. Absorption was measured at $OD_{436nm}$ and the enzyme activity was expressed as U $mg^{-1}$ protein.

### 2.4. Statistical Analyses

Two-way ANOVA was used to analyse the data in GraphPad Prism 8.0. Tukey's test was used to compare treatments conducted in this study at $p<0.05$.

## 3. Results

### 3.1. Volatile Organic Compounds (VOCs) Emission

The compound emitted from rice leaves was analysed using SPME-GCMS. A total of 68 VOCs were obtained from the four treatments, of which 38 VOCs were from drought stress treatment (inoculated with PGPR or non-inoculated) (Table 1). In contrast, 30 VOCs were produced from irrigated conditions either with PGPR or without PGPR (Table 2).

Under drought conditions, it has been determined that ethanol is the dominant compound emitted from non-inoculated rice (T1), whereas 3-Hexen-1-ol is the main compound emitted from inoculated rice (T3).

Under non-stress conditions, Furan tetrahydro-3-methyl-4-methylene- exhibits as the highest emitter among non-inoculated (T2) rice samples, whereas the compound 2-Hexenal exhibits as the highest emitter among inoculated rice (T4).

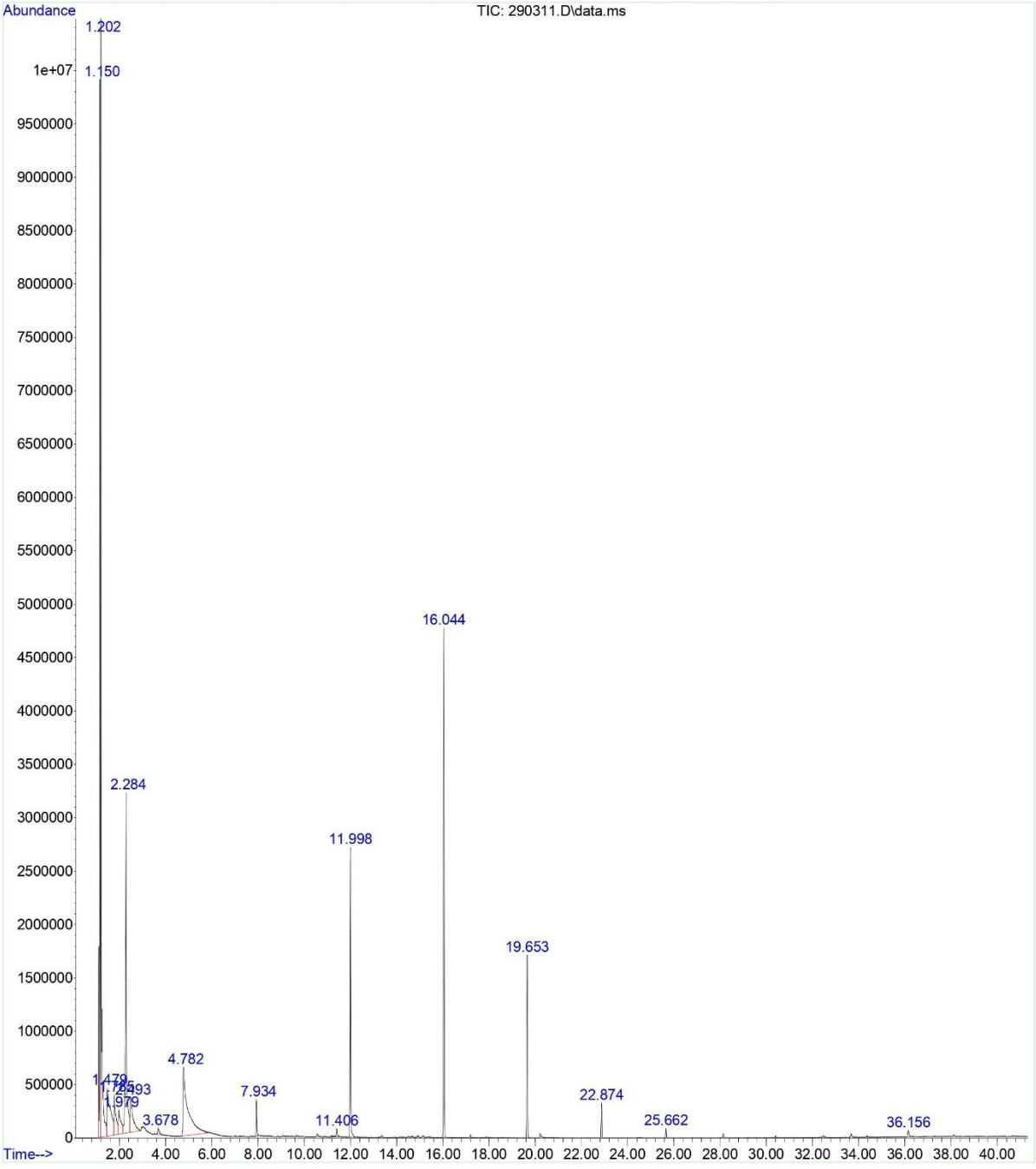
However, at 16.0 minutes (Figure 1), a high peak was detected in both non-inoculated (T1, T2) and inoculated (T3, T4) treatments in drought and non-stress conditions. The highest peak demonstrated the presence of Dodecamethylcyclohexasiloxane, which is not a natural product from leaves but is believed to be a contaminant from the fibres used in the analysis tools. In addition, other compounds such as Decamethylcyclopentasiloxane and Tetradecamethylcyclopentasiloxane, which were detected at 12.0 and 19.6 minutes, respectively, are also derivatives of polydimethylsiloxane fibres (PDMS) used in this method as described by Grimm et al. [13]. In leaf samples taken under drought stress, VOCs produced by T1 (non-inoculated) numbered up to 14 (Table 1) compared with non-stress, which produced 11 compounds (T2: non-inoculated) (Table 2). Rice inoculated under drought (T3) had 18 compounds (Table 1) compared with inoculated rice (T4) under non-stress which produced 13 compounds (Table 2).

**Table 1.** Retention times $R_t$ [min] of compounds obtained in rice leaves under drought conditions for inoculated and non-inoculated plants

| **Compound** | **Rt (min)** | **non-inoculated (T1)** | **inoculated with PGPR (T3)** |
|---|---|---|---|
| Ethylene oxide | 1.15 | 14.79 | 4.32 |
| Ethanol | 1.20 | 20.08 | - |
| Acetone | 1.26 | 6.24 | - |
| Butane | 1.26 | - | 2.44 |
| 1,3-Butanediol, (S)- | 1.39 | - | 1.01 |
| Propanal, 2-methyl- | 1.39 | - | 0.85 |
| Ethanone, 1-oxiranyl- | 1.48 | 5.77 | - |
| 2-Butanone, 3-methyl- | 1.48 | - | 1.33 |
| Methyl glyoxal | 1.53 | 3.25 | - |
| Silver acetate | 1.60 | - | 1.11 |
| Butanal, 3-methyl- | 1.78 | 2.57 | 2.19 |
| 1-Penten-3-ol | 1.98 | 2.05 | 2.5 |
| 2-Penten-1-ol, (E)- | 2.09 | 3.21 | - |
| Acetoin | 2.25 | - | 4.23 |
| Propanoic acid | 2.28 | 12.32 | - |
| Ethanol, 2-nitro- | 2.29 | - | 3.22 |
| 1-Butanol, 3-methyl- | 2.49 | 3.5 | - |
| Vinyl ether | 2.49 | - | 2.19 |
| 1-Butanol, 3-methyl- | 2.49 | 3.5 | - |
| 2-Pentyn-1-ol | 2.78 | - | 0.63 |
| 2-Penten-1-ol, (Z)- | 3.00 | 3.21 | 2.92 |
| 4-Pentenal, 2-methyl- | 3.51 | - | 1.65 |
| Pent-4-enylamine | 3.51 | 1.29 | - |
| 4-Pentenal, 2-methyl- | 3.52 | - | 1.65 |
| 2-Hexenal, (E)- | 4.71 | 5.36 | 6.02 |
| 3-Hexen-1-ol, (Z)- | 4.79 | 11.72 | - |
| 3-Hexen-1-ol | 4.77 | - | 22.49 |
| Benzaldehyde | 7.71 | - | 0.3 |
| **Total VOCs produce** | | **14** | **18** |
| **Similar VOCs produce** | **4** | | |
| **Different VOCs** | | **10** | **14** |

**Table 2.** Retention times $R_t$ [min] of compounds obtained in rice leaves under non-stress conditions for inoculated and non-inoculated plants

| **Compound** | $R_t$ **(min)** | **non-inoculated (T2)** | **inoculated with PGPR (T4)** |
|---|---|---|---|
| Acetaldehyde | 1.16 | 0.82 | 1.28 |
| 1,3-Butanediol | 1.39 | - | 0.69 |
| 3-Aminopyrrolidine | 1.48 | - | 1.47 |
| Oxirane, 2,3-dimethyl-, cis- | 1.49 | 0.99 | - |
| Ethylamine | 1.56 | 1.7 | - |
| Butanal, 3-methyl- | 1.79 | 1.82 | 0.64 |
| 1-Penten-3-ol | 1.98 | 1.53 | 0.82 |
| Pentanal | 2.09 | 1.43 | - |
| Furan, 2-ethyl- | 2.09 | - | 7.39 |
| 2-Pentanol, 3-methyl- | 2.25 | 3.91 | - |
| (E)-2-Butenylcyclopropane | 2.79 | - | 0.72 |
| 2-Penten-1-ol, (Z)- | 3.02 | 1.86 | 5.26 |
| 3-Hexenal | 3.49 | 1.3 | 3.08 |
| 2-Hexenal, (E)- | 4.71 | 2.45 | - |
| 2-Hexenal | 4.73 | - | 31.44 |
| Furan, tetrahydro-3-methyl-4-methylene- | 4.78 | 9.18 | - |
| 2,4-Hexadienal, (E,E)- | 6.34 | - | 1.44 |
| trans-3,4-Dimethyl-2-hexene | 7.77 | - | 0.72 |
| Benzaldehyde | 11.18 | - | 0.88 |
| **Total VOCs produce** | | **11** | **13** |
| **Similar VOCs produce** | **4** | | |
| **Different VOCs** | | **7** | **9** |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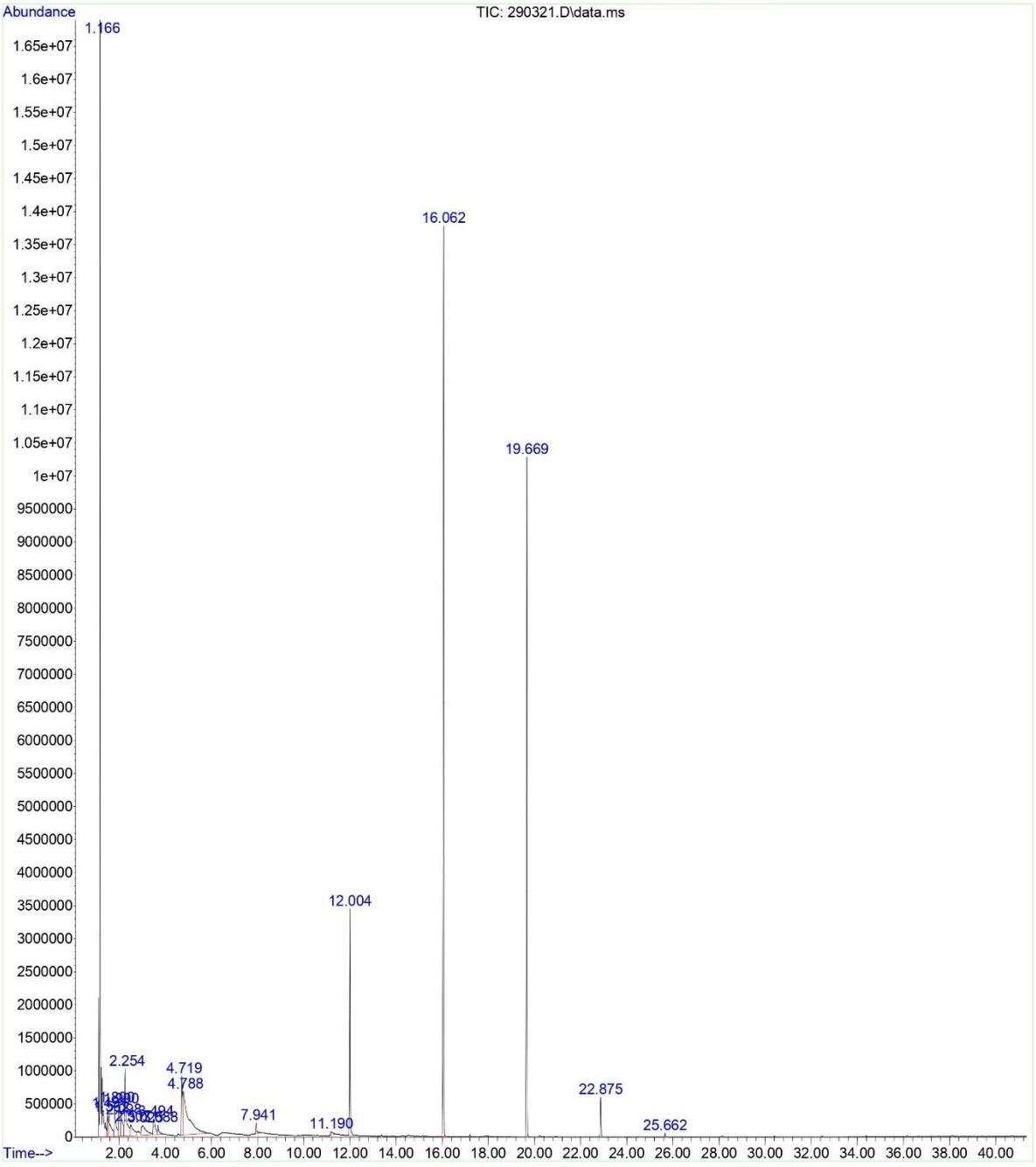
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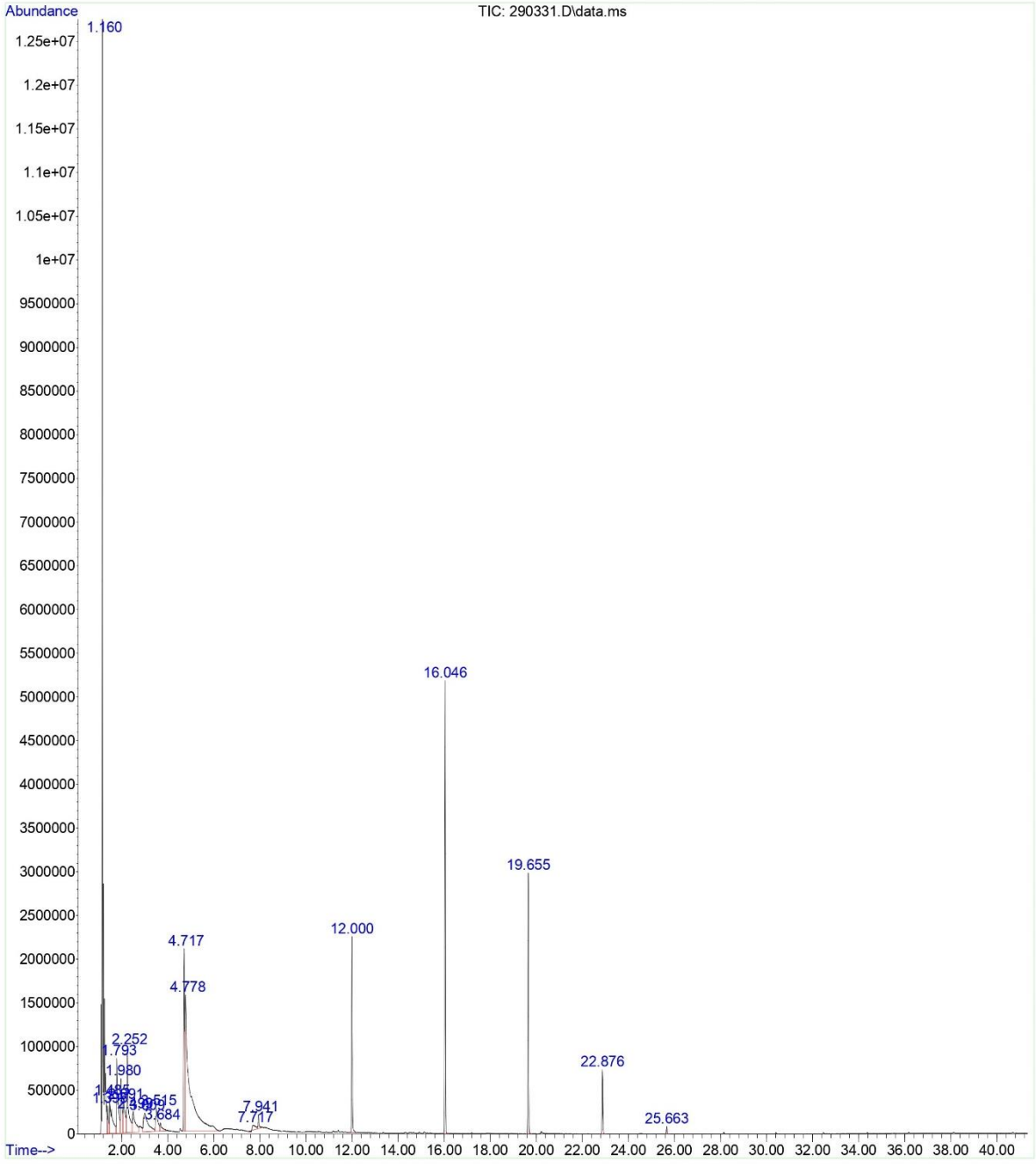
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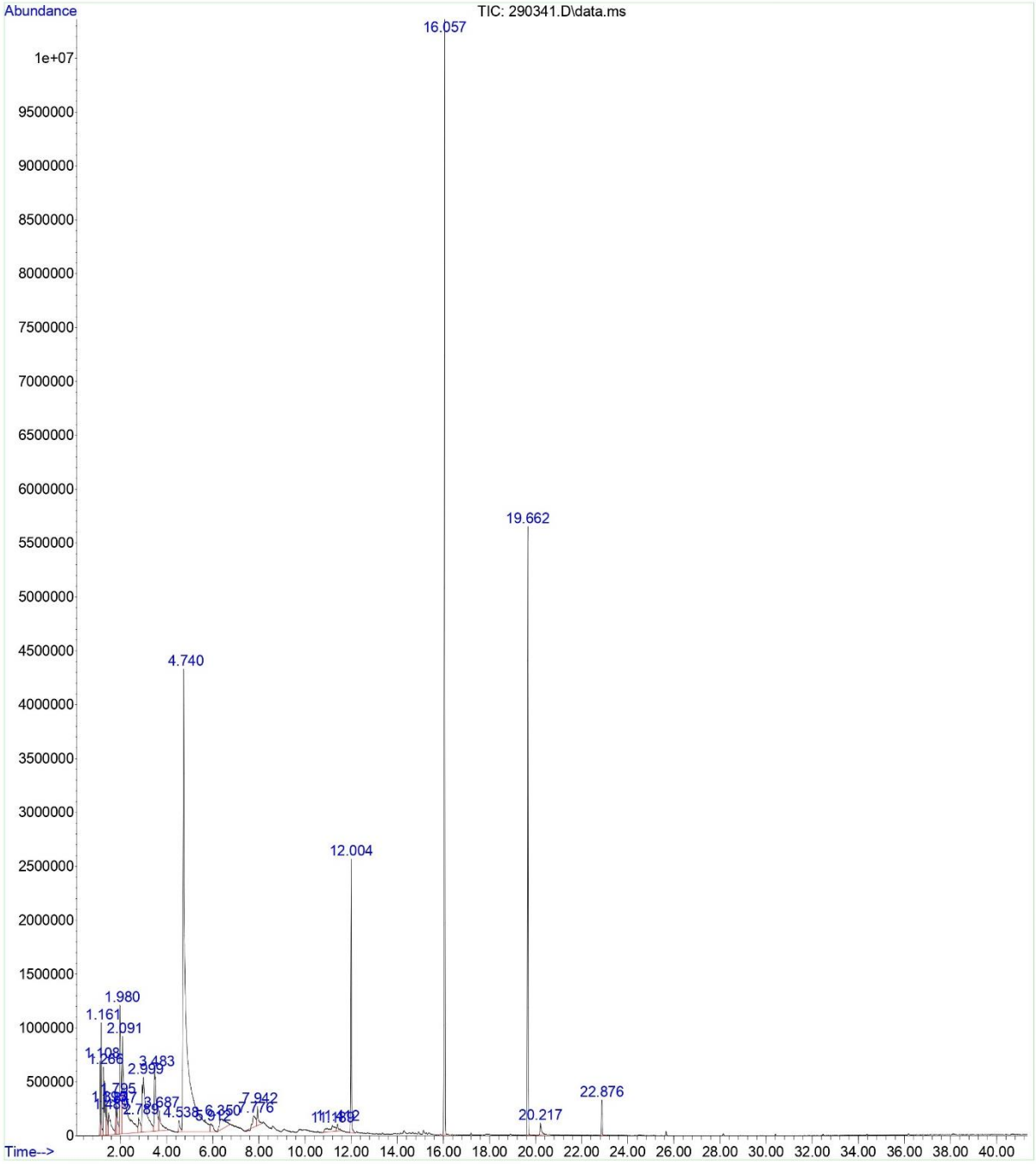



d)

**Figure 1.** Comparison of volatile organic compounds (VOCs) emitted by a) rice plants under drought stress – non-inoculated (T1), b) rice plants under non-stress conditions – non-inoculated (T2), c) rice plants under drought stress – inoculated with PGPR (T3) and d) rice plants under non- stress condition – inoculated with PGPR (T4)

For leaf samples taken under drought stress conditions (either non-inoculated (T1) or inoculated with PGPR (T3)), as shown in Table 1, the earliest minutes indicated the presence of EO instead of ethylene. EO is a portion of oxidised ethylene under the ethylene pathway reaction. The concentration of ethylene gas is higher than in non-stress conditions. However, in this study, the oxidised ethylene known as EO was detected only in drought treatments. The EO gas was the first known compound detected at 1.15 minutes, followed by ethanol, acetone and others in that order. Under drought stress, the EO content in T1 (non-inoculated) was higher at 14.8% than in T3 (inoculated) at 4.32%.

Other VOCs identified were alcohols such as ethanol, 1, 3-butanediol, (S), 1-Penten-3-ol, 2-penten-1-ol (E)-, ethanol, 2-nitro, 1 -Butanol, 3-methyl-, 2-Penten-1-ol, (Z)-, 3-Hexen-1-ol, (Z)-and 3-Hexen-1-ol. The aldehydes detected were ethyl oxide, acetone, butane, Propanal-2-methyl-, Ethanone, 1-oxiranil, Butanal, 3-methyl, 2-Hexenal, (E) and benzaldylhid. The ketones identified were 2-Butanone, 3-Methyl, methyl glucose, acetoin, 3-Hexenal-1-ol, (Z)-and 3-Hexen-1-ol, while the ester group consisted of Butanal, 3-methyl and 1- Butanol, 3-methyl.

Under the non-stress conditions (Table 2), the aldehydes identified were acetaldehyde, Oxirane, 2-3-dimethyl-cis-, 3-Hexenal, 2-Hexenal, 2-Hexenal, (E)-, 2, 4-Hexadinal, (E, E)-and Trans-3, 4-dimethyl-2-hexen. The alcohols detected were 1, 3- Butanediol, 1-Penten-3-ol, 2-Pentanol, 3-Methyl-, and (E) -2-butanylopropane. The ketones identified were pentanal, whereas the ester group consisted of Butanal, 3-methyl. All types of VOC, aldehydes, alcohols, ketones and esters, are commonly produced by rice leaves, where aldehydes are the most abundant.

The terpene compounds from rice leaves comprised of butanal, 3-Methyl-, 1-Penten-3-ol and 2-Penten-1-ol, (Z)-. All treatments produced some green leaf volatile compounds (GLV) in drought conditions. The resulting GLVs are 2-hexenal I, 3-Hexen-1-ol (Z) and 3-Hexen-1-ol. Under non-stress conditions, 2-Hexenal I was produced.

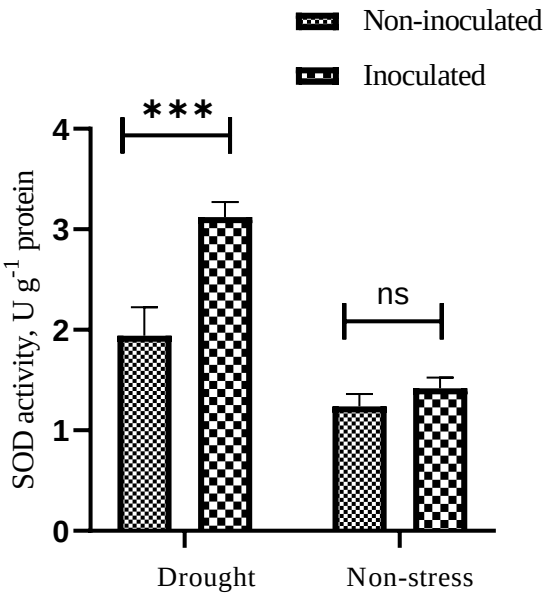
### 3.2. Enzymatic Antioxidant Activities

The antioxidant enzymes of SOD, CAT and GPX were evaluated for comparing inoculated and non-inoculated rice plants in both conditions, under drought stress and non-stress (Figure 2).

Inoculation with PGPR treatments significantly increased enzymatic activity in drought-stressed rice leaves (Fig. 1(a)). SOD activity was higher in inoculated rice leaves (drought and non-stress) than in those not inoculated. Based on the findings, inoculating PGPR into stressed rice plants improved SOD activity even under drought conditions. These findings showed that SOD is essential for a plant's initial defence mechanism as a ROS restorer.

In Fig. 2(b), inoculated rice produced higher CAT activity than non-inoculated rice under drought and non-stress conditions, although not significant under non-stress conditions.

The results (Fig. 2(c)) revealed that the rice plants treated with the PGPR consortium exhibited significantly elevated glutathione peroxidase (GPX) activity compared to the untreated plants.

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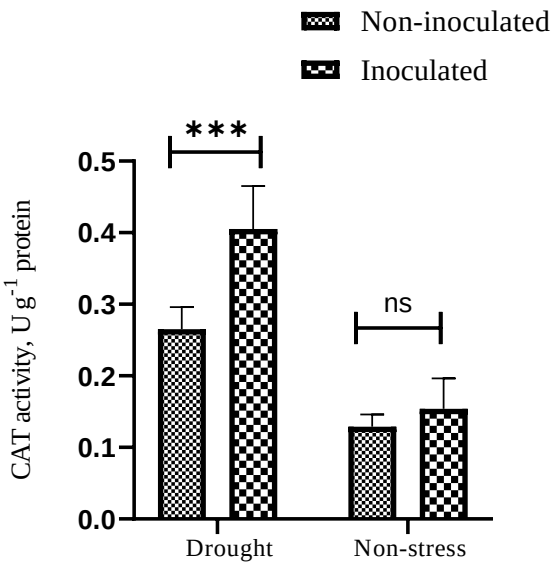


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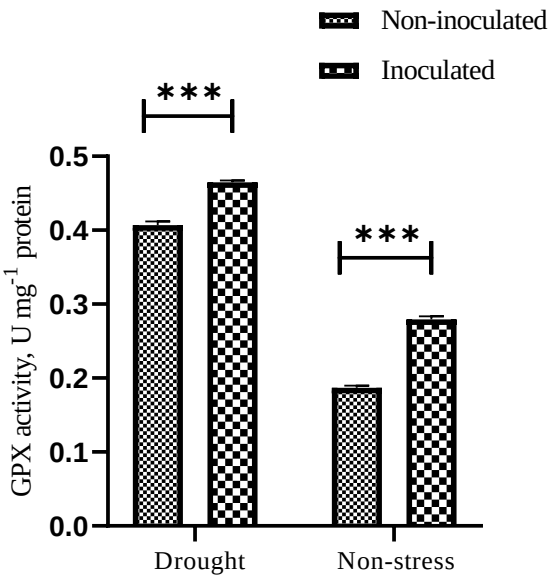


c)

**Figure 2.** The effect of drought stress on the activity of a) superoxide dismutase (SOD), b) catalase (CAT) and c) guaicol peroxidase (GPX) in rice leaves with and without PGPR consortium treatment. The data presented is the mean of the standard deviation (± SD) and an asterisk (*) indicates a significant difference where $P<0.05$ while ns = no significance.

# 4. Discussion

Rice plants subjected to drought stress and under irrigated conditions were examined for their response to treatment with a PGPR consortium. Plants naturally release VOCs through their flowers, leaves, and roots. Some PGPR strains produce VOCs that promote increased plant biomass, disease resistance, and abiotic stress tolerance [14]. Spinelli [15] reported that plants will usually produce more VOCs under stress conditions than non-stressed plants as part of their adaptation or response to stress. At present, very few studies on VOCs on inoculated rice in drought conditions have been conducted.

Plant biologists have discovered small amounts of gas in plant biological systems. They believe it plays an essential role in plant tissues and is detected as one of the ethylene metabolism products [16]. The rate of ethylene metabolism is easily detectable and has been observed in various plant tissues where ethylene represents the first biochemical process of ethylene action in plants. Based on the theory of ethylene synthesis [17], the conversion of 1-aminocyclopropane-1-carboxylate (ACC) substrate to ethylene is due to ACC oxidase. According to Smith et al. [16] and Wink et al. [18], ethylene can be degraded by oxidation to EO or ethylene monooxygenase and subsequently to ethylene-glycol. 1-aminocyclopropane-1-carboxylic acid (ACC)-synthase catalyses the last step. ACC oxidase converts 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene [19]. Thus, the generation of EO gas, as determined by the analysis, indicates that there was no occurrence of oxide reaction during the SPME extraction process [20]. However, EO is not a pathway to control endogenous ethylene concentration because only a small amount of ethylene can be metabolised to EO [16]. However, because only a small amount of ethylene can be metabolised to EO, EO is not a pathway to control endogenous ethylene concentration. As a result, it is unclear whether the EO obtained in this present study is related to the ethylene biosynthesis of plants under drought stress or vice versa.

Microorganisms produce two VOCs from inoculated rice from drought stress: acetoin and benzaldehyde [21]. These compounds will likely be obtained from microorganisms found in the rice leaves.

The presence of terpenes in inoculated rice under optimal non-stress conditions impacts the overall chlorophyll levels found in rice leaves. Terpenes possess the ability to alleviate abiotic stress in plants. A group of terpenes comprising 3-methyl-butanal, 1-penten-3-ol, and (Z)-2-penten-1-ol are among the VOCs rice frequently produces to aid photosynthesis. Plants terpenes serve as secondary metabolites and exhibit the ability to mitigate ROS [22]. GLV is released when mechanical damage and biotic stress occur on plants to provide immediate signals and information for most organisms found in the plant environment [23].

In this study, 1-Penten-3-ol and 2-Penten-1-l, (Z)- are the terpene group VOCs emitted from non-inoculated and inoculated rice plants under drought and non-stress conditions. Losses of VOCs are uptake by the plant itself. These losses can occur by adsorption on the cuticle and uptake through the stomata [24]. Uptake of VOCs through stomata requires a lower concentration of the compounds in the stomatal cavity than in the surrounding air. This concentration difference is vital because gases move along the concentration gradient inside and outside the leaf. The stomatal cavity is covered by water. Therefore, VOCs dissolved in water and metabolised in plant tissues can maintain a continuous uptake potential [25]. Ethylene is not involved in maintaining stomatal closure, and the ethylene decrease is probably a result of the inhibition of ACC synthase [26]. Some ethylene dioxide becomes EO, as described previously.

Plants activate antioxidant enzymes as the main destroyers of ROS to fight oxidative damage. Several studies [27] have shown that PGPR may play a role in reducing oxidative damage caused by abiotic stress by controlling antioxidant enzymes in different plants. Inoculation with PGPR induces plants to increase the activity of antioxidant enzymes such as SOD, CAT, and GPX under stress conditions. The inoculation with PGPR helps to reduce reactive oxygen species (ROS) accumulated in plants under stress [28].

In this study, the reduction in CAT activity under drought stress conditions in non-inoculated rice crops and non-stress inoculated crops was due to free radical

scavengers causing a decrease in $H_2O_2$ levels insufficient to activate the antioxidant properties of the enzyme. CAT is a key enzyme that can directly remove $H_2O_2$ and is essential for ROS detoxification during stress [29]. Drought stress inhibits CAT and GPX activities, but the activity of these enzymes is significantly higher in the presence of PGPR. Peroxidase activity increased in rice inoculated with PGPR under droughts and watered conditions (Fig. 1(c)). The study by Basu et al. [30] and Ashokkumar et al. [31] demonstrates that GPX can decrease the levels of $H_2O_2$ in cells under stress conditions. Antioxidant enzymes are found around plant cells to perform defence-related actions in conditions of activated oxidative stress [29]. The profiling of the VOCs in inoculated and non-inoculated rice plants under stress should be highlighted, particularly in this country. Ashokkumar et al. [31] have observed the VOC profile of eight rice varieties from South India to determine the differences in the aroma. From this study, the production of VOCs from plants can serve as a guide for assessing the status of plants immediately, whether they are under stress or not.

The study's findings indicate that PGPR inoculation effectively enhances crops' management stress. However, a more in-depth investigation of VOCs is needed because understanding the different types of VOCs produced can benefit the better and more efficient use of PGPR in crops. In contrast, antioxidant activity can verify the mechanism that occurs during stress.

Inoculation with PGPR induces plants to increase antioxidant content in drought stress conditions, which is the basis for reducing $H_2O_2$ accumulated in inoculated plants compared to plants without PGPR treatment [26].

Plants have very effective antioxidants and osmolytes that work together to control oxidation and protect plant cells from oxidative damage [32]. PGPR can synthesise antioxidant enzymes in response to stressful conditions. The PGPR-acquired stress signal acts as a stimulus for additional mechanisms, which can indirectly increase antioxidant activity in stressed plants. Therefore, inoculation with PGPR helps increase rice's antioxidant activity in rice plants under drought and also in irrigated conditions.

## 5. Conclusions

This study successfully determined the presence of several compounds in inoculated and non-inoculated rice leaf samples using solid-phase microextraction (SPME) and gas chromatogram-mass spectrophotometry (GCMS). Through this method, compounds produced by rice leaves are affected by the environmental conditions, such that inoculated rice under drought stress releases more than non-inoculated rice, producing several different compounds in different environmental conditions. Besides, inoculation with PGPR influenced the antioxidant enzyme activity of inoculated and non-inoculated rice under drought and irrigated conditions. The increase in antioxidant activity in inoculated rice (drought and non-stress) shows the influence of PGPR to improve rice defence against reactive oxygen species (ROS) and stimulate growth in drought conditions. Future research suggests that PGPR applications can be conducted in open areas to evaluate the effectiveness of PGPR in the field.

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## Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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