BIOLOGICAL AGENTS AND THEIR ROLE TO INCREASE PLANT ESSENTIAL OIL UNDER WATER STRESS

Agen Hayati dan Peranannya dalam Meningkatkan Minyak Atsiri Tanaman pada Kondisi Cekaman Air

AGUS PRAYITNO KURNIAWAN1, NURUL AINI*, MOCH. DAWAM MAGHFOER1, WIWIN SUMIYA DWI YAMIKA1, and RESTU RIZKYTA KUSUMA2

1 Department of Agronomy, Faculty of Agriculture, Universitas Brawijaya.
Jl. Veteran Malang 65145, East Java, Indonesia
2 Department of Plant Pest and Diseases, Faculty of Agriculture, Universitas Brawijaya.
Jl. Veteran Malang 65145, East Java, Indonesia
*Email: nra-fp@ub.ac.id

ABSTRACT

Essential oils are plant natural products resulting from secondary metabolites used for raw materials of various industries such as perfumery, preservative, cosmetics, and pesticide. The major problem of essential oil plants cultivation is the low essential oil content. Enhancing essential oil content is one of the main focuses in developing essential oil plants which can be reached by water management. Growth and yield reduction and changes in some physiological reactions are the responses of a plant toward water supply shortage (water stress). Water stress triggers elicitors and some signal molecules produced for secondary metabolites resulting in higher essential oil percentages. However, it would also decrease essential oil yield following lower biomass production. Some microorganisms can produce phytohormone and enhance nutrient uptake allowing the plant to cope under water stress condition. Understanding how the environment affected plant secondary metabolite (especially essential oil), as well as microorganism roles for crop production, will provide proper cultivation technology to increase plant essential oil content and oil yield. This review aimed to analyze the potential use of some biological agents to alleviate the negative effect of water stress on essential oil plants.

Keywords: cultivation, microorganism, secondary metabolism

INTRODUCTION

One of plant natural products is an essential oil, a secondary metabolic stored in leaves, stems, flowers, and roots of the plant. In Indonesia, the total export value of essential oil was 185 million USD in 2019, making it one of the leading exports commodities (Anonymous, 2021). Indonesia has a high diversity of essential oils plants such as patchouli (Pogostemon cablin Benth), palmarosa (Cymbopogon martin Stapf), citronella (Cymbopogon winterianus Jowit), pepper (Piper nigrum L.),...
nutmeg (*Myristica fragrans* Houtt.), vetiver (*Vetiveria zizanioides* Stapf) and sandalwood (*Santalum album* L.) (Sankarikutty and Narayanan 2003). Patchouli oil is one of the best essential oil due to its fragrant qualities and its unique aromatic which are difficult to be substituted (Anonis 2007). The essential oils utilization is mostly for various industries products such as antiseptic, perfumery, pesticides, and cosmetics.

Essential oils belong to the terpenoid group, being synthesized through MVA (cytoplasmic mevalonate) and MEP (methylerythritol 4-phosphate) biosynthesis pathways with the precursors of Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (Enfissi *et al.*, 2005). Sesquiterpenes were supplied precursors from the MVA pathway, while hemiterpenes, monoterpenes, and diterpenes were supplied from the MEP pathway. In patchouli, the biosynthesis of patchouli sesquiterpene alcohols occurs in specific organelles, the highest content in the leaves, and was lower in the stems and flowers (Tang *et al.*, 2019). The essential oils were accumulated in a particular site in special structures such as trichome glands and the leaves’ internal structure. The trichomes number was correlated positively with the sesquiterpene accumulation. Furthermore, glandular trichomes were located in the adaxial and abaxial epidermis. The glandular trichomes found in the stems and leaves were termed internal glands, they were also located in the hairy surfaces of leaves and stems (Guo *et al.*, 2013). Rusydi *et al.*, (2013) observed patchouli plant tissue under a light microscope and scanning electron microscope and discovered six glandular trichomes and two non-glandular trichomes. Patchouli alcohol was the highest component found in patchouli essential oil and the other 24 types of components belonged to sesquiterpene (Sugimura *et al.*, 2005; Paul *et al.*, 2010).

Low essential-oil yield is still the major problem in essential oil plant cultivation. Water management may be the alternative way to optimize essential oil yield (Sangwan *et al.*, 2001). Plants require sufficient water to support their growth and development. A higher plant essential oil content had been reported under water stress conditions, but there was a decrease in oil yield accumulation due to biomass reduction (Agami *et al.*, 2016). To obtain the optimum cumulative oil production, microbial approaches such as the utilization of biological agents (Plant Growth-Promoting Rhizobacteria/PGPR and Arbuscular Mycorrhizal Fungi/AMF) can be applied. The evaluation of PGPR (*Pseudomonas chlororaphis*) and AMF (*Glomus etunicatum, G. interradices*, and *G. mosseae*) indicated their ability to support plant growth under biotic stress like water deficit through phytohormones production mechanism and antioxidant activity enhancement (Brilli *et al.*, 2019; Al-Arjani *et al.*, 2020). Therefore, this review aimed to analyze the potential use of some biological agents for alleviating the negative effect of water stress on essential oil plants.

**THE ESSENTIALITY OF WATER IN PLANT GROWTH**

Water affects all stages of plant growth, playing an important role in various biochemical reactions of plant metabolism and transporting nutrients and organic compounds. Occupying 80-85% of the biomass, water allows herbaceous plants to grow straight by keeping their turgid pressure (Fagerstedt 2009). Polarity and viscosity properties support the water role as solvent and transporter allowing the capillarity movement through plant tissue. Water also has certain thermal properties, cooling the plant tissue when the ambient temperature is too high (Araya and García-Baquero 2014).

Transpiration is the process of releasing water into the atmosphere where most of the water (90%) is absorbed by plants. Transpiration begins with raising soil water to the roots, then transmitted to the leaves through xylem tissue in the stem, and finally, evaporate into the atmosphere (Chavarria and dos Santos 2012). Water flows through cell walls, cell membranes, and air spaces during the transpiration process. The water flows through osmosis, diffusion, and mass flow. Osmosis is water flow through a semi-permeable membrane (cytoplasmic membrane of a cell). On the other hand, diffusion is water flow due to different solutions (high to low). Finally,
mass or bulk flow is the water movement under external pressure, resemble to form a gradient (Kowles, 2010; Fricke, 2017).

Soil plays a vital role in plant life. Water can be stored in the soil for supplying plant requirements in all growth stages. Soil water storage is a dynamic property that can be referred to several conditions. Field capacity (FC) depicted the water amount that can be detained by the soil through its capillaries, after saturation and gravity drainage. Permanent wilting point (WP) indicated the water volume per soil weight that was unable to be absorbed by roots. It has a certain matrix density and the plant was incapable to maintain turgor pressure causing plant wilt. By considering soil volume and root exploration, the available water content (AWC) for plants can be estimated according to FC and WP values. The AWC value was calculated through the difference between FC and WP (Chavarria and dos Santos 2012). FC is often used for determining the water amount given in the context of water management.

RESPONSE OF ESSENTIAL OIL PLANT TO WATER STRESS

In some periods, soil water availability sometimes is unable to suffice water plant requirements. This condition is often marked as water stress. Over the past several years, the extreme drought condition caused a great impact on food stability in the world. Growth inhibition and photosynthesis rate decline were the main effect of physiological aspects affected by water stress (Osakabe et al., 2014). This effect is related to the limited nutrients absorbed by plants (Bista et al., 2018). Water stress mostly affected the photosynthesis aspect in plant physiological reactions (Chaves et al., 2009). Based on the intensity and duration period, water stress can be categorized into severe, moderate, and low. Yield reduction has occurred in various plants. Severe water stress during the vegetative phase caused higher plant height and biomass reduction (up to 30.21%) while a larger reduction in seed yield (up to 48.69%) was obtained from stress during reproductive stage (flowering and seed filling phase) of dill (Anethum graveolens L.) (Ghassemi-Golezani et al., 2008). Water stress caused physiological and morphological changes in plants. Some species of essential oil plants gave different responses to water deficits. A 70% of irrigation treatment based on water requirement (estimated from evapotranspiration) decreased plant fresh weight of Thymus capitatus, Lavandula latifolia, and Mentha piperita. Furthermore, the dry weight of L. latifolia and the oil content of L. latifolia and Salvia sclarea also decreased due to the treatment (García-Caparrós et al., 2019).

Various responses of plants due to water stress also affected plant anatomy. Agami et al., (2016) described anatomical structures changing in basil plants under water stress conditions, through reductions in xylem vessel thicknesses, vascular cylinders, cortex, and pith diameter. Meanwhile, anatomical changes in leaf organs can be observed from the diminution in palisade, blade, and spongy tissues thicknesses; anatomical structure through decreasing in stomatal density, stomata dimensions, and stomata opening areas, oil glands number, but increased the glandular trichome density.

Under water stress circumstances, plants produced an excessive level of ROS (reactive oxygen species) formed as O$_2^-$, $\cdot$O$_2^-$, H$_2$O$_2$, and OH caused oxidative stress (Mattos and Moretti, 2015). Moreover, water stress reduced the plant yield, caused photosynthetic inhibition, induced alteration in chlorophyll content and chlorophyll composition, damaged the photosynthetic organ and enzymes activities related to antioxidant and carbon metabolism processes (Muller et al., 2011). Essential nutrients were dissolved and transported to the root by water. Thus, water stress would cause nutrient imbalance, reduced nutrient diffusion, and dissolved nutrient-rich-water (magnesium, sulfates, nitrates, calcium, etc.) mass flow (Rouphael et al., 2012).

The water stress effect can be observed on plant natural products (secondary metabolites), such as essential oil. Stress conditions stimulate secondary metabolites biosynthesis through the production of elicitors or signal molecules (Ramakrishna and Ravishankar 2011). Water stress reduced leaf area, causing a higher density of oil glands resulting in a greater amount of essential oil accumulation (Abdi et al., 2019). Nik
et al., (2008) reported that despite lower plant height and lower biomass produced in *Parthenium argentatum* plants, essential oil percentages increased and its chemical constituents were different quantitatively. Similarly, Sarmoum *et al.*, (2019) reported that three different water treatments: tap water (TW), non irrigated (NIR), and salt water (SW) on rosemary applied at the flowering initiation stage until the flowering stage (three weeks). The result showed that TW and NIR treatments produced significantly higher essential oil percentages and oil yield than SW.

Reducing water supply will induce the production of higher secondary metabolite products and alter their constituents while their primary growth was inhibited. A summary of the water stress effects on some essential oil plants was shown in Table 1.

**Table 1. Effect of water stress treatments on some essential oil plants**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Water Stress Treatments</th>
<th>Effect</th>
<th>References</th>
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<tr>
<td><em>Sage</em> (<em>Salvia officinalis</em>)</td>
<td>Irrigation based on field capacity percentages (%) [25 (severe), 50 (Moderate) 100 (control)]</td>
<td>Fresh and dry matter reduction respectively under moderate (35.33 and 17.85%) and severe stress (74.6 and 26.33%) compared to control. Increasing essential oil content 1.77% under moderate and 1.01% under severe stress compared to control. Essential oil constituents, ketones (camphor, α-thujone, β-thujone) and ether (1.8-cineole) increased up to 267.44 and 452.12% under moderate compared to control.</td>
<td>Bettaieb <em>et al.</em>, (2009)</td>
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<td><em>Thymus carmanicus</em></td>
<td>Irrigation based on soil water depletion percentages (%) [80 (severe) 50 (moderate) and 20 (no stress)]</td>
<td>Fresh and dry weight of the plants were lower under moderate and severe stress. Drought stress resulting higher essential oil content. Under moderate stress, EO content (%) was higher at 12.5% while under severe stress it was higher at 44.9%. In addition, EO yield (kg ha⁻¹) was lower at 43% under moderate stress and 44.1% under severe stress.</td>
<td>Bahreininejad <em>et al.</em>, (2014)</td>
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<td><em>American basil</em> (<em>Ocimum americanum</em>) and Sweet basil (<em>O. basilicum</em>)</td>
<td>Irrigation based on % FWC (field water capacity) (125, 100, 75, and 50)</td>
<td>O. <em>americanum</em> showed higher total fresh and dry weight at 50% FWC (drought) and 125% FWC (excessive water), whereas at 75% FWC indicated the highest fresh and dry weight. 50% FWC (drought) and 125% FWC (excessive water) produced in higher essential oil content (%) while 100 and 75% FWC contained higher essential oil yield (g plant⁻¹)</td>
<td>Khalid (2006)</td>
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<tr>
<td><em>Marigold</em> (<em>Calendula officinalis</em>)</td>
<td>Irrigation based on % field water capacity (FWC) (25, 50, 75, and 100)</td>
<td>Plant irrigated with 75% FWC had the highest flower yield. The highest essential oil percentage at all flowering stages was obtained from plants treated with 25% FWC.</td>
<td>Metwally <em>et al.</em>, (2013)</td>
</tr>
<tr>
<td><em>Parsley</em> (<em>Petroselinum crispum</em>)</td>
<td>Three levels water deficit : level 1 (30-45%), level 2 (45-60%), control (0-10%)</td>
<td>Water deficit (level 1 and 2) reduced plant growth (leaves number, fresh weight of foliage and root) as well as increased essential oil content (40-120%) and oil yield (3-17%) at plain-leaf and curly-leaf cultivars.</td>
<td>Petropoulos <em>et al.</em>, (2008)</td>
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**THE ROLE OF BIOLOGICAL AGENTS IN INCREASING ESSENTIAL OILS PLANT’S GROWTH AND YIELD UNDER WATER STRESS**

Some living microorganisms in the rhizosphere which interact with plant roots could increase plant growth through their metabolic activities. Some bacteria can adapt well in stressed environments, such as drought and salinity. Bacteria stimulates plant structures modification, to induce their tolerance to stress conditions, such as drought or water stress.

Some bacteria which colonized plant roots naturally and stimulate the growth of the host plant is termed Plant Growth Promoting Rhizobacteria (PGPR). PGPR application in agriculture aims to enhance growth rate, increase biomass production, and improve plant yields.
**Satureja hortensis**

Four irrigation treatments. Control (100% FC during growing season (FC)), Moderate stress 1 (66% FC during vegetative stages (LS 1)), Moderate stress 2 (66% FC during flowering stages (LS 2)), Severe stress (33% FC during vegetative stages (HS)).

HS treatment reduced plant fresh and dry weight by 50 and 56% respectively, whereas LS 1 was by 46 and 51%. HS significantly increased oil concentration 31.42% compared to FC, whereas LS 1 and 2 were 14.28% higher than FC.

**Anise (Pimpinella anisum L.)**

Greenhouse experiment:
Irrigation at 20, 40, 60, 80, and 100% of available water (AW).

Field experiment:
No irrigation during stem elongation (I₀), No irrigation during stem elongation and grain filling stages (I₁), no irrigation during umbel appearance (I₂), no irrigation during grain filling period (I₃), and fully irrigation in all growing stages (I₄).

At the greenhouse experiment, growth (leaf area, SLA, RWC, RGR) and grain yield were decreased at 20 and 40% AW, increasing essential oil percentage but decreased essential oil production (mg pot⁻¹). At the field experiment, water treatment I₀, I₁, and I₂ reduced biomass and grain yield. I₁ and I₂ produced the highest essential oil content 2.63%, whereas essential oil yield at I₀ and I₃ was >20 kg ha⁻¹.

**Rosemary (Rosmarinus officinalis)**

Soil moisture regimes
(Irrigation water (IW): cumulative pan evaporimeter (CPE)) at 1.00, 0.75, 0.50 and 0.25.

Herbage and oil yield increased at soil moisture regime 0.5 compared to 0.25, whereas higher soil moisture regimes (0.75 and 1.00) were not significantly different.

**Balm (Melissa officinalis)**

Irrigation 100, 80, 60, 40, and 20% of field capacity.

Water deficit started at 60% FC, increased essential oil content 39-189% compared to 100% FC. Irrigation 60% FC produced higher essential oil yield compared to 100% and 80% FC up to 92.71 and 68.88%, respectively. Meanwhile, 40% FC reduced essential oil up to 41.01%.

**Lemongrass (Cymbopogon citratus)**

Soil moisture depletion at % total available water (TAW) (20, 30, 40, 50, 60, and 100).

Soil moisture depletion under 100% TAW reduced shoot fresh and dry weight, whereas soil moisture depletion at 40-60% of TAW resulted in a higher yield of essential oil 32-43% compared to 100% TAW.

**Patchouli (Pogostemon cablin Benth.)**

Four levels of soil water content (100, 75, 50, and 25% FC).

Vegetative growth of the plant was reduced at 50 and 25% FC. Essential oil content was increased linearly following soil water content reduction. At Patchouli 1 variety, the highest essential oil yield was obtained at 100% FC (2.02 ml plant⁻¹). Whereas at Sidikalang variety and Tetraploid Patchouli, essential oil yield was not different significantly.

Notes: SLA: specific leaf area; RWC: relative water content; RGR: relative growth rate.

The beneficial effect of PGPR is closely related to the role of the bacteria in growth hormones synthesis (IAA, cytokinin, and gibberellin). Another advantage of PGPR is the capability of bacteria to release volatile organic compounds (VOC) which was gas molecules that can interact with roots in the soil environment (Santoro et al., 2015). Induction of systemic resistance bacteria in Arabidopsis thaliana could increase plant growth (Ryu et al., 2004). VOC can also play roles as electron donors or acceptors in metabolic reactions. In vitro research by Banchio et al., (2009), showed that plants exposed to Bacillus subtilis GB03, increased fresh biomass and essential oils yield of sweet basil (O. basilicum), and showed higher α-terpineol and eugenol as primary components of basil oil 0.2 and 10 times respectively compared to control. In another research, by releasing the volatile compound 2R,3R-Butanediol, the application of Pseudomonas...
Arbuscular mycorrhizal fungi (AMF) were used to enhance plant growth and nutrient absorption. In basil plants, shoot and root dry weight, nutrient uptake, essential oil content and oil yield increased due to the application of *Glomus fasciculatum*, followed by *G. etunicatum* and *G. intraradices* (Rasouli-Sadaghiani et al., 2010). The increasing growth and essential oil content were also reported in *Oregano* (*Origanum sp.*) as the result of *Glomus mosseae* application (Khaosaad et al., 2006). Furthermore, the inoculation of *Rhizophagus clarus* and *Claroideoglomus etunicatum* enhanced plant height, root length, and essential oil compositions of *Piper aduncum* (de Oliveira et al., 2019). In addition, microscopic analysis of roots revealed the presence of hypha, vesicles, and arbuscules in the inoculated plant. The effectiveness of AMF used was greatly affected by fungi species and their compatibility with the host plants. Arpana et al., (2008) evaluated the effect of some AMF species (*Gigaspora margarita, G. fasciculatum, G. intraradices, G. leptotichum, G. mosseae, G. bagyarajii, G. macrocarpum, G. monosporum Acaulospora laevis, and Scutellospora calospora*) to patchouli. The highest growth and patchouli oil content were obtained from *G. etunicatum* application among other AMF species. Syafruddin et al. (2021) also reported that dual mycorrhizal genus application (*Glomus mosseae + Gigaspora sp.*) enhanced patchouli essential up to 47% compared to single mycorrhiza application.

In essential oils plants, environmental conditions such as water supply had a high impact on essential oil production. The application of PGPR was reported to increase plants' tolerance toward water stress (Brilli et al., 2019). Agami et al., (2016) revealed that PGPR (*Pseudomonas fluorescens, P. mendocina, Azotobacter chroococcum, and Azospirillum lipoferum*) application was able to alleviate the negative effects of water stress on basil plants. PGPR application through seed soaking increased fresh and dry weight of shoot, fresh and dry weight of root, and oil content. Water use efficiency (WUE) and electrolyte leakage (EL) were higher on plants exposed to water stress. However, PGPR played a role in increasing WUE and reducing EL. PGPR was also enhanced antioxidant activity of basil essential oil.

*A. lipoferum* + 150 kg N ha⁻¹ treatment, followed by no bacteria + 150 kg N ha⁻¹ (0.97%) and *Azospirillum* + 150 kg N ha⁻¹ (0.96 %) treatments (Dastborhan et al., 2012). PGPR with organic manure application, have a positive effect on the essential oil content. The combination of *Azotobacter chroococcum* and *Bacillus megaterium* with compost manure produced the highest essential oil content of *Rosmarinus officinalis* L. compared to the single NPK fertilizer treatment (Abdelaziz et al., 2007). PGPR species applied have different influences on plant growth. Ordookhani et al., (2011) evaluated several species of PGPR on basil. The result indicated that *P. putida, A. chroococcum*, and *A. lipoferum* application increased plant growth (fresh and dry weight of shoot and root), nutrient uptake, and essential oil content. The highest essential oil content was obtained from consortium application (*Pseudomonas* + *Azotobacter* + *Azospirillum*) compared to a single application. Furthermore, considering the essential oil quality, Ordookhani (2011) also reported that the inoculation such bacteria (*P. putida, A. chroococcum*, and *A. lipoferum*) increased microelement content (Zn, Fe, Mn, and Cu) represented the antioxidant activity of basil essential oil.

*clororaphis* O6 increased systemic tolerance of *A. thaliana* toward drought by producing some hormones (jasmonic acid, salicylic acid, ethylene, and abscisic acid) which could reduce stomatal openings (Cho et al., 2008).

For essential oil plants, the optimal and balanced nutritional fulfillment based on plant requirements and conditions becomes an important factor in determining the quality and quantity of essential oils produced. PGPR is a biofertilizer having a positive effect on improving plants' growth and yields, as well as reducing inorganic fertilizer. Interactions between some rhizobacteria (*Azotobacter chrocooccum, Azospirillum lipoferum*, and *Azotobacter* + *Azospirillum*) and nitrogen fertilizer dosages significantly affected the essential oils percentage of *Matricaria chamomilla* L. (medicinal and essential oil-producing plant). The highest essential oils content (0.99%) was obtained from *Azotobacter* and 0 kg N ha⁻¹ treatment, followed by no bacteria + 150 kg N ha⁻¹ (0.97%) and *Azospirillum* + 150 kg N ha⁻¹ (0.96 %) treatments (Dastborhan et al., 2012). PGPR with organic manure application, have a positive effect on the essential oil content. The combination of *Azotobacter chroococcum* and *Bacillus megaterium* with compost manure produced the highest essential oil content of *Rosmarinus officinalis* L. compared to the single NPK fertilizer treatment (Abdelaziz et al., 2007). PGPR species applied have different influences on plant growth. Ordookhani et al., (2011) evaluated several species of PGPR on basil. The result indicated that *P. putida, A. chroococcum*, and *A. lipoferum* application increased plant growth (fresh and dry weight of shoot and root), nutrient uptake, and essential oil content. The highest essential oil content was obtained from consortium application (*Pseudomonas* + *Azotobacter* + *Azospirillum*) compared to a single application. Furthermore, considering the essential oil quality, Ordookhani (2011) also reported that the inoculation such bacteria (*P. putida, A. chroococcum*, and *A. lipoferum*) increased microelement content (Zn, Fe, Mn, and Cu) represented the antioxidant activity of basil essential oil.
enzymes activities (Polyphenol Oxidase, Peroxidase, and Catalase). Similar to PGPR, the AMF application also aimed to alleviate the negative effect of water stress. Hazzoumi et al., (2015) reported that the application of G. intraradices on Ocimum gratissimum, stimulated plant growth and oil yield in water stress conditions. Furthermore, Hazzoumi et al., (2017) stated that AMF was successfully mitigating diameter reduction and extracellular shrinking spaces of the trichome gland and keeping its essential oil content in the glands. It caused higher essential oil content of O. gratissimum inoculated by AMF than uninoculated plant under water stress conditions. Summary of the effect of AMF and PGPR on some essential oil plants under water stress were shown in Table 2.

The application of PGPR could be combined with AMF. Compare to the single application method, parallel application of both PGPR and AMF resulted in higher effectiveness to stimulate plant growth and enhance plant biotic (water, salinity, and heavy metal) and abiotic (pathogens) stress tolerance. As reported by Marulanda et al., (2006), G. intraradices (drought-tolerant AMF) combined with Bacillus thuringiensis resulted in maximum root growth as well as enhancing nodules number, mycorrhizal colonization, plant growth, and water absorption of Retama sphaerocarpa (legume) exposed to water stress. PGPR and AMF species affected the effectiveness of their combination. The proper combinations showed well compatibility and synergy in plant growth stimulation. Awasthi et al., (2011) reported the evaluation of some AMF species (G. fasciculatum, G. mosseae, G. intraradices, G. aggregatum,) and some species of PGPR (B. subtilis and Stenotrophomonas sp.). The higher plant growth and yield of artemisinin were obtained from G. mosseae + B. subtilis treatment than control and other PGPR + AMF treatments. The beneficial effect of the AMF + PGPR combination in reducing the negative effect of water stress was triggered by bacteria’s role in improving fungal development under drought conditions (Vivas et al., 2003).

Simultaneous inoculation of AMF and PGPR was also reported effective to improve plant and essential oil yield. Higher geranium (Pelargonium graveolens) fresh biomass, 89% in the first year and 92% in the second year was obtained from Bacillus subtilis (PGPR) and Glomus mosseae (AMF) combination treatment, compared to a single application of G. mosseae (75% and 85%), and B. subtilis (32% and 39 %). Essential oil content was not affected by inoculation of G. mosseae and B. subtilis, but the increasing biomass resulted in a significantly higher essential oil yield of geranium (Alam et al., 2011). Meanwhile, Arpana et al., (2009) reported that a combination of G. etunicatum and A. chroococcum, Methylobacterium mesophilicum, Burkholderia cepacia, and Trichoderma harzianum increased both of biomass and essential oil content of patchouli (Pogostemon cablin Benth.). Biological agents (PGPR and AMF) take a role as biostimulants and biofertilizers alleviating the negative effect of water supply shortage. It can be obtained by determining the optimum point of reduced water quantity to achieve the maximum value of essential oil content and oil yield.  
Tabel 2.

Table 2. Effect of PGPR and AMF on some essential oil plants under water stress

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<tr>
<th>Plant</th>
<th>Biological Agents</th>
<th>Water conditions</th>
<th>Effects</th>
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<tr>
<td>Satureja hortensis</td>
<td>Pseudomonas fluorescens (strain PF-135 and PF-108)</td>
<td>Well-watered and 50% field capacity (FC)</td>
<td>Under water stress circumstances, inoculation of PF-135 increased essential oil content up to 25.45%, while inoculation of PF-108 decreased essential oil content up to 25.92% compared to the uninoculated plants. Inoculation of G. intraradices under non-stress conditions resulted in the highest essential oil yield in the first and second yields 37.5 and 66.13 kg ha⁻¹ respectively.</td>
<td>Mohammadi et al., (2017)</td>
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<td>Glomus mosseae (GM), Glomus intraradices (GI), and G. mosseae + G. intraradices (GM+GI)</td>
<td>No water stress (Control (D1)), No irrigation during stem elongation-flower initiation (D2), No irrigation at the flower initiation (D3), and No irrigation at 50% flowering-full flowering (D4)</td>
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<td>Zakerian et al., (2020)</td>
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<tr>
<td>Species</td>
<td>Bacterial Strains Used</td>
<td>Experiment Conditions</td>
<td>Essential Oil Content Increase</td>
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<tr>
<td>Lemongrass (Cymbopogon citratus)</td>
<td><em>Pseudomonas</em> sp. and <em>Azotobacter</em> sp.</td>
<td>Irrigation based on % FC (100, 75, 50, and 25)</td>
<td>Under 75% FC, essential oil content increased, while water stress below 75% FC decreased essential oil content. <em>Pseudomonas/Azotobacter</em> applications increased essential oil yield by 14% compared to control. Application of <em>S. rimosus</em> and <em>S. monomycini</em> enhance the fresh and dry weight of shoot and root of plants exposed and not exposed to water stress either at greenhouse or field experiment. The essential oil content of peppermint increased due to the application of <em>S. rimosus</em> and <em>S. monomycini</em> both in once and twice inoculation on plants exposed and not exposed to water stress at greenhouse up to 15.90%. Water stress altered essential oil components such as menthol, piperitone, and isomenthone improvement up to 33, 30, and 23%. EO components were also increased by all bacterial inoculation treatments.</td>
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<tr>
<td>Peppermint (Mentha piperita)</td>
<td><em>Streptomyces rimosus</em> strain and <em>S. monomycini</em> (once and twice inoculation)</td>
<td><strong>Greenhouse experiment:</strong> Non-stress and water stress (60% FC) started 30 days after planting</td>
<td>Field experiment: Non-stress and water stress (50% FC) started 30 days after planting</td>
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<td>Thymus daenensis</td>
<td><em>Pseudomonas fluorescens</em> + <em>Pseudomonas aeruginosa</em></td>
<td>Non stress (well-watered), irrigation depleted 20-25% of FC (low stress), 35-40% FC (mild stress), 55-60% FC (severe stress)</td>
<td>Water stress decreased dry weight of shoot and root, then application of PGPR enhanced dry weight of shoot and root. Low water stress increased essential oil content as much as 14.81% while severe water stress reduced essential oil content up to 25.92%. PGPR treatments showed no effect on essential oil content.</td>
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<td>Thyme (Thymus vulgaris)</td>
<td><em>Rhizophagus intraradices</em> + <em>Funneliformis mossae</em></td>
<td>Non stress (well-watered), irrigation depleted 20-25% of field capacity (low stress), 35-40% FC (medium stress), 55-60% FC (severe stress)</td>
<td>Water stress reduced shoot and root fresh and dry weight. Under medium and severe stress, AMF inoculation increased shoot fresh weight and dry weight. Water stress enhanced essential oil content in both control and mycorrhiza inoculations up to 13.58% and 19.27% respectively. The presence of AMF resulted in greater essential oil content at medium water stress up to 26.92%.</td>
<td></td>
</tr>
<tr>
<td>Chamomile (Matricaria chamomilla)</td>
<td><em>Pseudomonas fluorescens</em> strain PF-135</td>
<td>Well-watered and water deficit (50% FC)</td>
<td>Root dry weight, flower fresh and dry weight, and also shoot dry weight were reduced under water deficit treatment, inoculation of PF-135 enhanced them on both well-watered and water deficit conditions. Water deficit increased essential oil content both in non-inoculated and inoculated plants. A higher increase was found at non-inoculated plants (approximately 71% and 6%). On the other hand, bacteria inoculation enhanced essential oil yield approximately 128% at well-watered conditions.</td>
<td></td>
</tr>
</tbody>
</table>

**Biological Agents and Their Role to Increase Plant Essential Oil Under Water Stress** (AGUS PRAYITNO KURNIAWAN et al.)
CONCLUSION

Optimizing oil production of some essential oil plants can be achieved through water management and biological agents utilization such as Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhiza Fungi (AMF). Aromatic plants which produce essential oil from their secondary metabolism are valuable crops to be cultivated and traded in many countries. Improvement both in quantity and quality of essential oil, lead to the development of environmental and microbial technologies.

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