



Effects of PGPR, AMF and *Trichoderma* Applications on Adaptation Abilities to Different Biotic and Abiotic Conditions in Medicinal and Aromatic Plants

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ABSTRACT

Medicinal and aromatic plants are valuable sources of herbal products worldwide due to their secondary metabolite content, high antioxidant activities and many other biological activities. As a result of the developing technology, the demand for natural active substances obtained from plants has increased. For use, plants collected from nature do not have the desired quality standards. For this reason, sustainability can be achieved by using microbial inoculants as well as many biotechnological and molecular approaches such as micro propagation, synthetic seed technology to increase the yield and quality standards of medicinal and aromatic plants. Thanks to microbial inoculants, yield increase can be realized and at the same time, product quality can be contributed due to increased soil quality. In this review, it was aimed to evaluate the important roles of plant growth promoting rhizobacteria (PGPR), Arbuscular mycorrhizal fungi and *Trichoderma* inoculants in increasing productivity, nutrient uptake and resistance of medicinal and aromatic plants to environmental stresses in the light of literature. In this review, the variation in the resistance of plants to environmental stresses is summarized by evaluating the ultimate effects of microbial inoculants alone and in combination. In addition, it has been added to the evaluation in studies to prevent the decrease of secondary metabolite content formed under environmental stress conditions in medicinal and aromatic plants by microorganisms.

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Introduction

Medicinal and aromatic plants are grouped according to their families, taxonomic categories, morphological and pharmacologic features, active ingredients, consumption and use, organs used and pharmacological effects. Most medicinal plants are endemic species and their medicinal properties are mainly due to the presence of secondary metabolites that respond to stimuli in natural environments and cannot be expressed under culture conditions (Figueiredo and Grelle, 2009).

Medicinal aromatic plants, most of which are endemic species, have increased their use worldwide due to their secondary metabolite content that responds to stimuli in natural environments (Cole et al., 2007).

Primary metabolites refer to compounds of nucleic acids, proteins, carbohydrates, fats and lipids. Primary metabolites are found in all plants and perform metabolic activities by participating in nutrition and reproduction (Croteau et al., 2000). Primary metabolism refers to the processes that produce the carboxylic acids of the Krebs cycle. Secondary metabolites, on the other hand, are not

essential for life, but contribute to the survival of the species (Pagare et al., 2015). Of the secondary metabolites, carotenoids and flavonoids play a role in cell pigmentation in flowers and seeds by attracting pollinators. Therefore, they also participate in plant reproduction (Winkel-Shirley, 2001).

Secondary metabolites are synthesized in specific ways by specific biosynthetic enzymes in tissues, organs, and growth parts of the plant. Although the functions of secondary products are differing, those which are cytotoxic against microbial pathogens, therefore they are used as antimicrobial agents in medicine (Briskin, 2000; Goldman, 2001).

Medicinal and aromatic plants encounter many problems arising from both biotic and abiotic stress conditions in the environment they grow due to the medicinal metabolites they contain. In a given environmental condition and on a selected genotype, agronomic factors deeply affect plant growth and quality. Pointed out that secondary metabolites are fundamentally

produced by genetic pathways, although their biosynthesis is strongly influenced by environmental factors (Yazdani and Jamshidi, 2002). Biotic factors are stress factors resulting from fungal, bacterial and viral infection of microorganisms and attacks by harmful animals. Abiotic factors are environmental factors such as water, temperature, radiation, chemicals, magnetic and electrical fields. (Lichtenhaler, 1996). This means that biotic and abiotic stress factors highly affect their growth parameter, essential oil yields and chemical composition. Among the abiotic environmental stress factors, salinity and drought have the most important effect on medicinal plants (Aziz et al., 2008; Heidari et al., 2008).

An abiotic stress reduces the uptake and diffusion of CO₂ and alters different biochemical reactions, which further inhibits photosynthesis (Flexas et al., 2004). These stresses are the prime causes of crop failure and decreasing the yield by more than 50% (Bray et al., 2000) and pressurize the sustainability of agriculture sector.

Microbial based bio stimulants have been crucial since the beginning of agriculture (i.e., *Rhizobium* in legumes) and current expectations include its commercialization as a complementary to crop nutrition. The beneficial effects of microorganisms to plants depend upon sophisticated nutritional and chemical signaling as well as soil and climate factors. Plant roots release sugars, organic acids, amino acids and phenolics, which affect the composition of rhizosphere communities, leading to beneficial relationships. (Ortiz-Castro et al., 2009).

The plant rhizosphere is a region of intense microbial activity where many microorganisms colonize on and around the roots of growing plants have an ecological importance. Various groups of bacteria, mycorrhizae and other groups of microorganisms are associated with the root systems of all higher plants. In studies on the interactions of beneficial microorganisms with the plant, it has been reported that depending on the plant and microorganism species and ecology, it may have either positive, negative, or neutral effects on host plant (Glick et al., 1999; Ertürk et al., 2008; Ertürk et al., 2010). Bacteria, one of the largest groups of microorganisms can promote plant growth by attaching to the outer surface of the plant, such as roots (rhizosphere) or leaves (phyllosphere), or by forming endophytic relationships on the inner surfaces of the plant. It has been suggested that PGPR can increase plant growth by reducing the ethylene level in the plant. In these cases, the immediate precursor of ethylene is 1 aminocyclopropane-1-carboxylate (ACC). This compound is hydrolyzed by bacteria expressing ACC-deaminase activity. The products of this hydrolysis, ammonia and α -ketobutyrate, are used as nitrogen and carbon sources by bacteria that break down the ACC (Glick et al., 1999).

Another group of microorganisms which are active in the plant rhizosphere are arbuscular mycorrhizal fungi. Mycorrhiza is a term derived from the Greek words mikes (fungus) and riza (root), meaning "root fungus". The term mycorrhiza refers to the cooperation or symbiosis between plants and fungi that colonize the cortex tissue of the roots during the active plant development phase. This cooperation is characterized by the flow of carbon, which is the production of plants to the fungus and the nutrients taken by the fungus to the plant. They interact with a wide variety of other soil organisms at the root or in the soil's

rhizosphere. Some form a symbiotic association and in turn alter the host physiology (Fitter and Garbaye, 1994). *Trichoderma* fungi can also be expressed in the fungal groups that are effective in the rhizosphere. They are endophytic saprophytes, readily colonize the root surface of the host plant, benefit plant health as biocontrol agents against various fungal pathogens and promote the development of plant growth. (Pandya and Saraf, 2010; Metwally and Al-Amri, 2019).

In this review, the effects of the use of PGPR, Arbuscular mycorrhizal fungi and *Trichoderma* inoculants in medicinal and aromatic plants on soil fertility, plant nutrient uptake and plant resistance to environmental stresses will be discussed in the light of the literature. Information will be given about the activities of microbial inoculants alone and in combination and the results of the researches carried out on the related species to date.

Plant Growth Promoting Rhizobacteria (PGPR)

In recent years, the use of microorganisms has gained importance to reduce the need for chemical fertilizers and for sustainable agricultural activities. Of these microorganisms, the group that includes plant growth promoting bacteria (PGPR) directly produces growth promoting substances (hormones, vitamins, enzymes, siderophores), inhibits ethylene synthesis, binds atmospheric nitrogen to the soil, improves iron, organic and inorganic solubility, phosphate. It also increases the resistance of plants to salinity, metal toxicity and pesticide damage. Also, as a bio-agent, it reduces the harmful effects of phytopathogenic microorganisms. It supports plant growth by increasing the solubility and mineralization of microbial metabolites and inorganic and organic phosphorus (Glick et al., 1999; Dobbelaere et al., 2003; Çakmakçı et al., 2006; Çakmakçı et al., 2007).

Medicinal and aromatic plant species are important because they are consumed without much processing and the harvested crop does not contain synthetic compounds. Excessive use of chemical fertilizers has negative effects on plant and soil health. Considering the environmental pollution caused by excessive use of fertilizers and high production costs; Rhizobacteria (PGPR), which is a plant growth promoter, can be used in sustainable agricultural production (Kutlu et al., 2019).

Optimal and balanced mineral fertilization adjusted to the nutritional requirements and growing conditions of medicinal aromatic plants is an important growing factor that determines the amount and quality of essential oil (Nurzynska-Wierdak, 2013). Although bacterial and herbal studies are usually focused on herbaceous plants, studies on medicinal plants are quite scarce. PGPR in medicinal and aromatic plants has been studied in the following plants *Origanum majorana* L. (Banchio et al., 2008), Rosemary (Leithy et al., 2006), *Pelargonium graveolens* (Mishra et al., 2010), sweet fennel (Rezvani Moghaddam et al., 2011), dill (Hellal et al., 2011), mint (Santaro et al., 2011), basil (Ordookhani et al., 2011), coriander (Hassan et al., 2012), Mexican marigold (Cappellari et al., 2013), *Thymus daenensis* (Bahadori et al., 2013), sage (Ghorbanpour et al., 2016), Turkish thyme (Kutlu et al., 2019).

Although some studies have addressed the role of PGPR added to medicinal plants (Santoro et al., 2011), it is slightly known about the effect of PGPR on the production of secondary metabolites (Banchio et al., 2008). Moreover, generally, the efficiency of rhizobacteria applications on medicinal plants may be limited or variable (Cappellari et al., 2015).

In a study conducted in Iran the impact of inoculating *Ocimum basilicum* roots with plant growth-promoting rhizobacteria (PGPR) on plant growth indices such as shoot wet weight, shoot dry weight, root fresh weight, root dry weight, shoot height, N, P and K content and essential oils has been evaluated. PGPR inoculants are *Pseudomonas putida* strain 41, *Azotobacter chroococcum* strain 5, and *Azospirillum lipoferum* strain. Compared to the control group, it was stated that all factors increased with maximum root fresh weight (3.96 g/plant), N content (4.72%) and essential oil yield (0.82%) of PGPR inoculants in *Pseudomonas* + *Azotobacter* + *Azospirillum* application. (Ordokhani, 2011).

Similar results were also obtained in the applications of different varieties of lemongrass with phosphate-solubilizing bacteria (*Pseudomonas putida* 41, *Azotobacter chroococcum* 5 and *Azospirillum lipoferum*). Therefore, it has been determined that the combined use of bacterial isolates provides increases in plant height and biomass (Ratti et al., 2001).

Hassanpour et al. (2012) reported that the growth and yield of the pennyroyal (*Mentha pulegium* L.) were decreased significantly under water stress, and that some PGPRs applications under stress conditions could increase the secondary metabolites and plant biomass.

In the study, on the *Satureja hortensis* L. PGPR and humic acid combinations applied under drought stress were able to eliminate many problems caused by drought stress or reduce the effect of stress to fair level. Especially the applications which can effectively provide significant increases for total biomass, essential oil yield and chlorophyll levels even under drought stress conditions was useful (Ghazvini et al., 2014). *Agrobacterium* sp., *Bacillus* sp., *Pantoea* sp. and *Pseudomonas* ssp. Three different bacterial bioformulations (F1, F2 and F3) have been prepared using a total of 19 bacterial isolates. The cloves of garlic (*Allium sativum* L.) dipped in these bioformulations were planted in pots and the effects of the treatments on plant height, chlorophyll level and some enzyme (catalase, peroxidase, polyphenoloxidase and superoxide dismutase) activities were determined.

It was observed that all bacterial formulation applications made significant contributions to plant growth in garlic and caused significant changes in plant enzyme levels compared to the control groups. (Esringü et al., 2016).

Previous studies have showed that rosemary (*Rosmarinus officinalis* L.) has antibacterial, antioxidant, antiviral and immune system enhancing effects on the amount of essential oil composition in the rosemary plant by promoting bacterial (*Pseudomonas fluorescens*) growth stimulation (Gachkar et al., 2007).

The effects of salinity stress on levels were investigated. Contrary to the decreases observed in essential oils in parallel with the increase in salinity levels, it was estimated that the essential oils could remain

constant even under the same dose of salinity stress with bacterial applications (Bidgoli et al., 2019).

Asgharia et al. (2020), investigated the effect of PGPR applications on the protection of pennyroyal (*Mentha pulegium* L.) against drought damage. For this purpose, single and combined uses of *Azotobacter chroococcum*, *Azospirillum brasilense* bacteria were performed. In different drought tests, PGPR inoculations significantly reduced the negative effects on low growth parameters and production of secondary metabolites caused by water stress. The results showed that bacteria could have different effects on their ability to improve plant properties and the combination of bacteria may be more effective for improving physiological and phytochemical parameters in pennyroyal.

The results of the studies show that the plant species, the ecology in which the plant grows, and the use of PGPR isolates singly or in combination may drive plant X PGPR isolate interactions.

Arbuscular Mycorrhizal Fungi Inoculation (AMF)

Endo-mycorrhizae are known as arbuscular mycorrhizae (AM). Their distinctive feature is that they form highly branched structures within the stem cortex cells. Mycorrhizal fungi generally multiply rapidly, both in the root and in the soil. Mycorrhizal relationship is generally observed approximately 92% of the plants in the world (Isaac, 1992). Today, mycorrhizae are divided into 7 different types: Vesicular arbuscular mycorrhizae, Ectomycorrhosis, Ectendomycorrhizae, Ericoid mycorrhiza, Arbutoid mycorrhizae, Monotropoid mycorrhiza, and Orchid mycorrhiza (Anonymous, 2021). Even if vesicular arbuscular mycorrhiza (VAM) and ectomycorrhizae (ECM) are the agriculturally important types, the arbuscular mycorrhizal (AM) symbiosis is probably the most widespread beneficial interaction between plants and microorganisms (Parniske, 2008). In several studies, it was reported that they play a crucial role in plant nutrition and growth in stressed conditions and enhance a few essential ecosystem processes (Siddiqui and Pichtel 2008; Nakmee et al., 2016).

These microorganisms are not only protecting plant roots against pathogenic organisms, but also protect the plant against heavy metal toxicity and salinity stress and increase the plant's resistance (Smith and Read, 1997). Thus, AMF applications on lemongrass (*Symbopogon martini*) and mint (*Mentha piperita*) increased the volatile fatty acid amounts and mineral nutrient contents compared to the control group (Qupta et al; 1990; Khaliq and Janardhanan, 1997). Based on the idea that mycorrhizal activity may show variable results in different medicinal aromatic plants, the effect of mycorrhizal symbiosis is also investigated in a total of 76 different medicinal aromatic plant species. Therefore, it was determined that the activity was higher in the vegetative growth stage of the plant than in the flowering and fruiting stages, and the interaction was occurred especially in parallel with a better root development. In addition, it was concluded that herbaceous plants showed more root colonization than shrub and branched plants, and mycorrhizal applications could be observed more clearly in herbaceous plants (Sadiq Gors, 2002).

Among the benefits which are provided by mycorrhizal applications, the increases in the number of seeds and umbrellas, balanced growth, and development, increases in essential fatty acids and positive changes in the chemical composition of the fennel plant have been demonstrated by many studies (Kapoor et al., 2004; El-Ghadban et al., 2006).

Pankaj et al. (2021), in their study on the determination of the effect of AMF colonization on seedling emergence and growth in saline soil, seeds of *Palmarosa* (*Cymbopogon martinii*) plant ('Tripta', 'PRC-1' and 'CIMAP-Harsh') were grown in pots inoculated with AMF (*Rhizophagus intraradices*, *Funneliformis mosseae*, *Glomus aggregatum* and *Rhizophagus fasciculatus*). They have reported that inoculation with AMF significantly improved seed formation, shoot and root development, and viability indices. They stated that *R. intraradices*, 'Tripta' cultivar was the most effective in improving seedling emergence, fresh shoot and root weight and viability index. They stated that AMF colonization in saline soils is suitable for growing healthy seedlings of 'Tripta' and *F. mosseae* varieties and can increase agricultural yield with high economic returns.

Jeet and Baldi. (2012) inoculated the Fennel plant (*Foeniculum vulgare*), a valuable medicinal plant, with Arbuscular mycorrhizal-like fungi (*Sebacina vermifera*), Phosphate-solubilizing bacteria (*Pseudomonas fluorescens*), Azotobacter (*Azotobacter chroococcum*) in their study. The response of individual microorganism species was evaluated with reference to essential oil emergence, plant growth and yield, along with the qualitative effects on the essential oil. They reported that *Sebacina vermifera* was relatively important in stimulating the emergence of fennel seeds, growing the plant and increasing the yield of essential oil. They stated that *Sebacina vermifera* has great potentials for its agro-ecological qualities in medicinal plant cultivation and crop production and increases phytochemical production in medicinal plants. They stated that *S. vermifera* application can increase all positive effects on plant performance and *S. vermifera* application may be a better alternative to traditional chemical-based fertilizers.

AMF affects nutrient and water uptake and cell regeneration as a result of root development and increased absorption capacity of the roots. Apart from phosphorus, it provides the intake of other nutrients such as nitrogen, calcium, copper, manganese, sulfur and zinc (Sieverding, 1991; Ortaş, 2002). *Arbuscular mycorrhizal fungi* establish a direct physical connection between their external hyphae and host plant and soil. Thanks to these hyphae, they provide not only the uptake of mineral ions from the soil to the plant, but also the uptake of carbon (Kapulnik and Douds, 2000c).

Moreover, they can provide better plant growth and reproduction at the soils which are poor about plant nutrients or inadequate moisture by obtaining the nutrients with extramatrical hyphae. As a result of all these benefits, mycorrhizal plants are generally more resistant to environmental stresses than non-mycorrhizal plants (Kapoor et al., 2004).

AMFs have positive effects on water usage and mineral matter intake as well as overall yield and quality parameters such as germination, biological yield, root

yield, essential oil content and essential oil yield and contribute to dry matter accumulation. In some studies, these plants were thought to be leading especially in the pharmaceutical and medicinal sectors. Therefore, to consider these activities, there is a need for future studies on the interactions of AMF, medicinal aromatic plant ecological conditions.

Trichoderma

Symbiosis takes place between crops and soil microorganisms, including plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF) which are considered as natural bio stimulants. *Trichoderma* spp. which belongs to a class of PGPF has been successfully used on a commercial scale for biological control of phytopathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Armillaria mellea* and *Chondrostereum purpureum* (Benítez et al., 2004; El-Komy et al., 2015).

The ability of *Trichoderma* about to sense, invade, and destroy other fungi has been a major trait behind their commercial success as biopesticides (Verma et al., 2007). Currently, more than 60% of all registered biopesticides contain a single *Trichoderma* isolate, or mixtures of *Trichoderma* species for greater bioactivity. The *Trichoderma* species are frequently used in biocontrol and the best studied regarding their mechanisms of action are *T. asperellum*, *T. atroviride*, *T. harzianum*, *T. virens*, and *T. viride*, and most of which also exhibit high biostimulant action on horticultural crops (López-Bucio et al., 2015). At the same time, because of quickly colonizing in plant roots and competing with phytopathogens for the place of infection or nutrients, *Trichoderma* fungi produce several substances as antibiotic properties and hydrolytic enzymes (cellulases, chitinases, xylanases, pectinases, β -1,3-glucanases, and proteases among others) through which they can. Numerous reports showed that several strains of *Trichoderma* have a significant reducing effect on plant diseases that are caused by soilborne and foliar pathogens (such as *Rhizoctonia solani*, *Phytophthora* spp., *Pythium ultimum*, *Fusarium* spp., *Alternaria alternata*, *Sclerotinia* spp., *Gaeumannomyces graminis*, *Tielaviopsis basicola*, *Verticillium dahliae*, and *Botrytis cinerea*) under greenhouse and field conditions (Harman et al., 2004).

Trichoderma spp. helps plants better resist to environmental stresses such as salinity and drought via reinforcing plant growth and reprogramming gene expression in roots and shoots. The fungal mycelium secretes different compounds that increase the branching capacity of the root system, thus improving nutrient and water acquisition. Therefore, the products of *Trichoderma* are used as biopesticide, biofungicide, bioinoculant, biostimulant, biofertilizer and plant growth promoter. *Trichoderma* is stronger in terms of capacity on carrying and uptaking soil nutrients and making them more effective and competitive than other soil microorganisms. By reducing the pH of the soil, it allows the uptake of phosphates, iron, manganese, and magnesium elements. Moreover, iron recovery is an important component at microbial competition, especially around the rhizosphere where microbial activities are intense. Also, the species of *Trichoderma* are gained popularity because of their

multifaceted efficacy among several microbial biofertilizers and biopesticides.

Application of *Trichoderma* inoculum at early stage of crop growth permits to maximize its benefits in terms of root development and nutrient uptake. Increases in plant growth following *Trichoderma* treatment depend on the specific crop or plant genotype. In a study conducted with *Trichoderma* species and *Salvia miltiorrhiza*, which are generally focused in horticultural plants, it was determined that the mycelium (EM) extract and polysaccharide fraction (PSF) were produced by *Trichoderma atroviride* D16 had effects on the growth and secondary metabolism of hairy roots. Furthermore, the results showed that the respective fungal isolate promoted hairy root growth and stimulated the biosynthesis of tanshinons (a biochemical substance extracted from *Salvia miltiorrhiza*, which has cytotoxicity to various tumor cells) in hairy roots. However, the comparison of efficiency of the distillates obtained with steam proves the positive effect of *T. asperellum* B35 on coriander. It is important that increasing the yield of essential oils does not mean a change in composition and this obtained oil is at the upper ranges of pharmacopoeial standards. At the same time, the usage of antagonistic fungi affects the improvement of biometric parameters of the plant. An increased yield and the number of coriander fruits are found to be at the level of ~60%. In the experimental combination with *T. asperellum* B35 in a study, it was reported that the biomass of the aerial parts of the plant was slightly reduced twice as large in isolation the accumulation of some phenolic acids (Ming et al., 2013). *Mentha arvensis* L. (Family *Lamiaceae*) was commonly called Japanese mint. In a different study, two strains of *Trichoderma* were tested for its effects on the growth and productivity of mint plants without using any chemical fertilizers. *Trichoderma harzianum* (NFCCI 2241) and *T. ovalisporum* (NFCCI2689) were isolated from soil samples of *Mentha* growing fields of CIMAP, Hyderabad. At the end, the herbal oil yield and menthol content were found to be the highest and a significant increase with NFCCI 2241 in both crops was occurred and this was more than with NFCCI 2689 (Ratnakumari et al., 2014).

In another study on coriander, biometric parameters and changes in volatile fatty acids were tried to be determined in plants treated with *T. asperellum* B35. The comparison of efficiency of the distillates which were obtained with steam, proved the positive effect of *T. asperellum* B35 on coriander.

Combined Applications of Microorganism Groups

In the study for investigating the symbiotic relationship between the inoculation of arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria in the roots of May chamomile (*Matricaria chamomilla*), an increase in the dominant active ingredients camazulene and bisabolene was reported in parallel with a 28% increase in essential oil yield with concomitant microorganism applications (Saedi- Farkoosh et al., 2011). Similarly, AMF (*Glomus intraradices*) and PGPR (*Bacillus polymixa*, *Pseudomonas putida*, *Azotobacter chroococcum*) applications were made in the South African origin stevia (sugar grass) plant, either alone or in combination. The results showed that

inoculation with a single microorganism significantly increased root and shoot biomass as well as stevioside, chlorophyll and NPK content in plants compared to control. However, such multiplying effects have been found to be significantly enhanced due to bicompatible inoculant mixtures resulting from their strong synergetic relationships among themselves. The present results also showed that among all plants inoculated in pairs, plants inoculated with AM fungi and PGPR had the maximum K content. In addition, it was determined that triple vaccination had more positive effects on chlorophyll a, b and total content than single vaccination, but the effects were less than double vaccination. Researchers have suggested that competition between microorganisms in triple inoculations reduced the effectiveness of inoculations (Vafadar et al., 2014).

Phytoremediation is the possibility of cultivation by different mechanisms in soils contaminated with heavy metals because of the abilities of some microorganisms and plants. In a study investigating the synergistic effects of plant growth promoting rhizobacteria (*Streptomyces*, *Azotobacter*, *Pseudomonas* and *Paenibacillus*) and arbuscular mycorrhizal fungi (*Glomus*, *Acaulospora* and *Scutellospora*) on the bioremediation of iron-contaminated soils, pearl millet daisy (*Pennisetum bihumorum*), it was explained that these microorganism groups were effective on the phytoremediation of soil which was contaminated with Fe^{+3} compared to control. The effects of concomitant use of AMF and plant growth promoting rhizobacteria species on plant growth also showed a higher phytoremediation efficiency than single usage. It has been stated that these might be due to the siderophore hormone that was produced by all microorganisms (Mishra et al., 2016).

The interaction of the use of AMF and *Trichoderma viride* at onions on biomass, total soluble protein, mycorrhizal colonization, amino acids, and phosphorus nitrogen content were investigated. Similarly, the combination of AMF and *Trichoderma* was caused a significant increase in total biomass, free amino acid, and soluble protein contents (Metwally et al., 2021). The potential of single or combined application of *Trichoderma harzianum* and *Stenotrophomonas* spp (Az-30), *Bacillus flexus* (Sd-30) and *Brevibacterium halotolerans* (Sd6) strains on growth and essential oil yield on Japanese mint (*Mentha arvensis*) was also evaluated. In vitro interaction studies were shown that it did not antagonize *Trichoderma harzianum* (Th) individually and didn't prove the possibility of using these combinations for plant growth. Furthermore, it was revealed that in vitro inoculation of *Trichoderma harzianum* and *Brevibacterium halotolerans* (Sd6) strains increased plant growth, oil content, petiole ratio, photosynthetic pigments, and nutrient uptake, and resulted in higher oil yield than other microbial treatments. The results demonstrated synergistic interactions between *Brevibacterium halotolerans* and *Trichoderma harzianum* while improving survival, plant growth and yield. It was determined that the same combination application had similar effects in field conditions (Singh et al., 2019).

Solanum viarum (Tropical soda apple) seedlings was evaluated based on the interaction between AMF (*Glomus aggregatum*), PGPR (*Bacillus coagulans*) and *Trichoderma harzianum*, maximum plant biomass of

combined uses of *G. aggregatum* + *B. coagulans* + *T. harzianum* (dry shoot and root weight-12.17 g). As a result the lowest biomass was recorded in the control and maximum percentage of maximum root colonization was seen *G. aggregatum* + *B. coagulans* + *T. harzianum* inoculated plants. Plant height (60.5 cm) was significantly increased in *G. aggregatum* + *B. coagulans* + *T. harzianum* triple inoculation and also the maximum number of spores was recorded in plants inoculated with *G. aggregatum* + *B. coagulans* and *G. aggregatum* + *B. coagulans* *T. harzianum*. Moreover, phosphorus, potassium, zinc, copper, manganese, and iron contents of leaf samples were reported to be highest in plants treated with *G. Aggregatum* + *B. coagulans* + *T. harzianum* as opposed to plants inoculated with *G. aggregatum* alone (Hemashenpagam, 2011).

Conclusion and Future Aspects

As a conclusion, especially, the possibilities of increasing or changing the growth, yield, essential oil content and components of medicinal and aromatic plants have not been extensively investigated using multi-specific bacteria.

The clear conclusion from the above considerations is that stressful environments can cause nutritional and hormonal imbalances in the cultivation of medicinal and aromatic plants resulting in a negative impact on plant growth and development. However, the stress-induced negative effect on plant growth can be mitigated and/or minimized by naturally occurring microorganisms such as PGPR, AMF and *Trichoderma*. Recently, proteomics-based techniques have opened an important door to reveal the molecular mechanisms of various abiotic stress responses. Several stress sensitive proteins are suggested for the plant using these techniques. Moreover, by using these proteins and their corresponding genes, it may be possible to produce stress-resistant medicinal plants soon.

Moreover, identification of genes which are controlled with stress tolerance traits in these microorganisms will also increase our knowledge of the molecular basis of stress tolerance mechanisms. Therefore, in many in vitro studies, it would be a positive step to register the relevant biochemical and physiological mechanisms of the stress factor. The early studies with microorganisms have also led to variable results in much information.

The areas that researchers should focus on might be turning to microorganism-based metabolite engineering in stressful environments, discovering which microorganism strains are beneficial for promoting plant growth, which should be used singly, and which should be used in combination, identifying target genes to promote growth under stress, and transferring target genes to plants through biotechnology (Shazad et al., 2015).

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