

The Effect of Plant Growth Promoting Rhizobacteria (PGPR), Salicylic Acid and Drought Stress on Growth Indices, the Chlorophyll and Essential Oil of Hyssop (*Hyssopus officinalis*)

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The side effects of chemical drugs and the human tendency to make greater use of natural products in order to keep their health as well as problems of modern medical system caused more attention of human to medicinal plants. Hyssop (*Hyssopus officinalis*) is a plant of the family Lamiaceae and is one of the most important medicinal plants containing essential oils. Despite of the other crops, medicinal plants are the plants that quality of materials compared to their quantity is much more important and necessary. Today using symbiotic microorganisms with plants as the bio-fertilizer for providing nutrients is considered. Among plant growth promoting rhizobacteria, *Azospirillum*, *Pseudomonas* and *Bacillus* can be pointed out. It was observed that using the bio-fertilizer Nitroxin and phosphate solubilizing bacteria on Chamomile increased vegetative yield, seed yield and essential oil yield. In order to study the effect of PGPR, salicylic acid and drought stress on growth indices, the chlorophyll and essential oil of hyssop, a factorial based on a completely randomized design with three replications was conducted at the research greenhouse, Faculty of Agriculture, ... University, during 2013- 2014. Treatments were drought stress, salicylic acid and PGPR. Results showed that drought stress reduced the growth and physiologic characteristics and increased essential oil percent and accordingly Hyssop essential oil yield increased. Also, salicylic acid caused the increase in the yield, physiologic and essential oil traits and avoided stress injuries and compensated decrease in growth traits. Growth promoting bacteria by producing some metabolites such as growth regulators, or types of vitamins as well as improved access to nutrients were directly increase plant growth and development. Among used bacteria, *Azospirillum* had the greatest impact on improving the measured traits.

Keywords: Medicinal plant- Hyssop- Drought stress- Salicylic acid- PGPR - Growth indices- Chlorophyll- Essential oil.

The side effects of chemical drugs and the human tendency to make greater use of natural products in order to keep their health as well as problems of modern medical system caused more attention of human to medicinal plants (Rahimzadeh *et al*, 2012); Therefore increasing the approach to using medicinal plants globally, doubled the importance of the cultivation and

production of these plants. At present, the demand for medicinal plants products as the usable products in health and medical industries is growing (Agha alikhani, 2011); so that the twentieth century is called as a century return to nature and the use of herbal medicines. Hyssop (*Hyssopus officinalis*) is a plant of the family Lamiaceae (Omidbaigi, 2007), and its aerial parts contain essential oils and phenolic compounds. Research showed Hyssop essential oil yield depends on the aerial parts biomass.

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On the one hand, plants face with a variety of biotic and abiotic stresses during growth stages. Water deficit is one of the most important abiotic stresses that reduce growth and yield in many crops and medicinal plants, especially in arid and semi-arid regions (Reddy, 2004). Despite of the other crops, medicinal plants are the plants that quality of materials compared to their quantity is much more important and necessary. So, in order to achieve the maximum quality, understanding the effective factors affecting the growth and development of medicinal plants is very important. Understanding environmental, vegetative and agronomic factors, play an important role in successfully cultivated medicinal plants (Ebrahimpur, 2007 and Omidbaigi, 2003).

One thing about medicinal plants and crops which seems to be important is crop improvement and providing nutrients requirements. Today using symbiotic microorganisms with plants as the bio-fertilizer for providing nutrients is considered and with the aim of using the potential of organisms and organic material in soil to increase the quantity and quality as well as maintaining the current bio-fertilizers, environmental safety have been common (Khase Sirjani *et al*, 2011).

One of the most important mechanisms of PGPR is plant hormones production that by make and release plant growth regulators, effective mechanisms will provide in biological control. These bacteria produce some substances which are called growth regulators that as secondary metabolites, they have direct and indirect effects on physiologic and growth stages of plants. These bacteria by producing metabolites such as plant growth regulators or vitamins as well as improved access to nutrients increase plant growth and development of plant (Kloepper, 1993). Also, the solubility of phosphorus by bacteria is one of the most important features of them for stimulating growth. These bacteria through the production of siderophores and hydrogen cyanide reduce the activity of plant pathogens or other microorganisms, and thus indirectly have a stimulating effect on plant growth (Alipur and Malakuti, 2011).

Among plant growth promoting rhizobacteria, *Azospirillum* (*Azotobacter* spp), *Pseudomonas* (*Pseudomonas* spp) and *Bacillus* (*Bacillus* spp) can be pointed out (Wani, 2010 and

Yao, 2010). These bacteria cause increasing growth and yield of important crops through various mechanisms such as reduction of plant pathogens, solve the insoluble phosphorus, siderophore production, synthesis of antibiotics and etc and cause stimulate plant growth (Ramezanpour, 2009; Ramezanpour, 2010 and Rashid, 2004.)

Drought stress by reducing leaf area, stomatal closure, reducing protoplasm activity and carbon dioxide fixation, causes reduction protein and chlorophyll synthesis and reduced photosynthesis (Imam, 2002). Drought by producing destructive products of reactive oxygen species such as superoxide, hydrogen peroxide, hydroxyl radical, organic hydroperoxides, singlet oxygen and perhydroxyl radicals causes the imbalance the formation of reactive oxygen species (Tale Ahmad and Haddad, 2008) which amount must be controlled in the cell. Reactive oxygen species are potentially has the potential to react with many compounds in cell and cause damage to the cell membrane and other vital macromolecules such as photosynthetic pigments, proteins, nucleic acids and lipids (Tale Ahmad and Haddad, 2008). According to the studies, along with the drought stress other stresses will happen which is included: reduce moisture available to the roots, increase evapotranspiration than water absorption, increase cellular respiration and damage metabolic and structural processes in cells to, light inhibition, light oxidation and eventually leaves death, increase harden of soil because of drying and effect on root growth, reduce leaves growth and photosynthesis, inaccessible nutrient in the root zone, the accumulation of salts in top layers of soil and around the roots and toxicity of nutrients (Kafi and Mahdavi Damghani, 2005)

Biotic and abiotic stresses cause specific defensive reactions in plant organs that develop during the plant evolution process. In addition to the structural defense mechanisms, chemical mechanisms occurs in response to stresses, that after a series of reactions, it cause the production of secondary metabolites (Pastirova, 2004). Salicylic acid or ortho hydroxy benzoic acid is phenolic compounds that there is in a large number of plants, this compound is known as a hormonal substance and has a essential role in the regulation of physiological activities such as growth, plant evolution, ion uptake, photosynthesis and seed

germination under salt and drought stresses (El Tayeb, 2005; Hassanzadeh, 2014, and Kang, 2003.)

Salicylic acid in plants is considered as a hormone-like regulator and it plays a role in defense mechanisms against environmental and biological stress. Induction of flowering, growth and development, synthesis of ethylene, opening and closing the stomata and respiration are important roles of salicylic acid (Raskin, 1992). Salicylic acid, in plants which they are under environmental stress, has a protective role. It increases the resistance to water deficit in wheat (Bezorkova, 2001). Some successful reports have been published on using this material in increasing the quantity and quality of medicinal plants (Ram, 1997). Ghraib (2007) studied the effect of salicylic acid on the two plants Basil (*Ocimum basilicum* L.) and Marjoram (*Origanum vulgare* L.) and the results indicated increase in plant height, number of (branch, node and leaf) per plant, leaf area, fresh and dry weight and total carbohydrates in salicylic acid (concentration 10^4 M). Also using salicylic acid (10^4 M) increased the quantity and quality of essential oil of basil and increase antioxidant activity.

In a study (Khalil, 2006) showed that using bio-fertilizers such as Azotobacter, significantly increased quantitative yield and active substances in Sand plantain (*Plantago psyllium*). Using Nitroxin bio-fertilizer and phosphate solubilizing bacteria on chamomile (*Matricaria chamomilla* L.) increased vegetative and seed yield and essential oil yield, but its effect was not significant on essential oil (Fallahi, 2009). Essential oil yield of Marjoram (*Origanum majorana* L.) also significantly increased by the Pseudomonas (Banchio *et al.*, 2008). In another study on Rosemary (*Rosmarinus officinalis* L.) which is done by (Leithy *et al* 2008), inoculation with Azotobacter increased essential oil percent. Using bio-fertilizers contain Azotobacter and Azospirillum in Common sage (*Salvia officinalis* L.) increased plant height and shoot fresh and dry weight (Vande Broek, 1999). Also the studies showed that the use of bacteria such as Azotobacter, Azospirillum and replace them with chemical growth regulators had a high efficiency on the growth and the essential oil composition of Common sage (Yousef *et al.*, 2004.)

According to the above, this research evaluates the role of salicylic acid and plant growth

promoting rhizobacteria on hyssop under drought stress.

MATERIAL AND METHODS

In order to study the effect of PGPR, salicylic acid and drought stress on growth indices, the chlorophyll and essential oil of hyssop, a factorial based on a completely randomized design with three replications was conducted at the research greenhouse, Faculty of Agriculture, Urmia University, during 2013- 2014. The geographical location of the city is Miandoab 46° and $6'$ East longitude and 36° and $46'$ North latitude and 1314 m altitude. The climate of the region based on the Köppen climate classification is climate with summer. Drought treatments included two levels of irrigation: normal (control) and water stress as the first factor, salicylic acid in two levels: control (non-use) and use 1 mM as the second factor and PGPR in four levels: control (non-inoculated), Azospirillum, Pseudomonas, Bacillus as the third factor. Used sand and soil before mixing separately were sterile three consecutive days for 4 hours at 120° C. After weighing each empty pots, bottom of pots with equall amount of coarse sand were filled (for drainage), then the samples of said soil were dried in the oven (at 100° C for 48 h) to determine the amount of moisture. Thus dry soil weight in pots was determined, soil texture was loamy clay. Bacterial inoculation was done according to rubbing seed method immediately before planting. After preparing the pots in each pot some seeds were sown and after emerging, plants were thinned in some stagee. A month after planting (plants had 6-8 leaves) pots were equally irrigated and from this point on, irrigation treatments were done by daily weighing pot and add water due to evaporation and transpiration (reduce the weight of each pots including plant). Foliar salicylic acid treatment were done in three stages, one month after the plants establishment in pots and then performed once a week in succession. Samplings from each treatment were performed under identical conditions. Fresh weight of the samples was measured by a digital scale. To determine the dry weight of the samples they were oven-dried (24 h at 45° C) before weighing. Finally harvested whole plant of each treatment was transferred to laboratory in order to determine the percent of

essential oil. Essential oil extraction was done by Clevenger and based on water distillation method, in the same situations for 3 hours. For extraction, 35 g of hyssop for each treatment with 50 ml of water and 1 g of chloride sodium (increase the boiling point and the complete extraction of essential oil) was added to the 500 ml flask (Humphrey, 1992.) Essential oil droplets in the water in two distinct phases moved to tubes moving graded and there due to lighter weight they accumulated on the water. In order to determine the percent of essential oil weight method was used, so that the small clean glass container in which had reached to a constant weight, weighed. Then the samples contain essential oil which was separated from Clevenger was transferred to the rotary evaporator. Then container containing the essential oil weighed again and the difference between the initial weight (weight of the glass) and secondary (glass weight and essential oil) amount of essential oil were obtained which were presented in weight percentage in 100g dry weight (Omidbaigi, 2006).

Statistical analysis of data was carried out with SAS (ver 9.1) and comparisons were made using Duncan's multiple range tests at $P < 5\%$. Also Excel software was used for drawing the diagrams.

RESULTS AND DISCUSSION

Results analysis of variance (Tables 1 to 3) showed that the effect of drought stress was significant on all traits. Also, the effect of salicylic acid similar to drought stress was significant on all traits. The effect of inoculation growth promoting bacteria was significant on plant height, fresh weight and dry weight, chlorophyll b, total chlorophyll and yield and essential oil percent and was not significant on relative water content, chlorophyll a and ratio of chlorophyll a to b. The interaction between drought stress and salicylic acid had a significant effect on plant height, weight dry, chlorophyll b and total chlorophyll and did not have any significant effect on the other traits. The interaction between drought stress and growth

Table 1. Analysis of variance studied traits

| S.O.V | df | Plant height (cm) | Shoot fresh weight (g) | Shoot dry weight (g) | Relative water content |
|--|----|----------------------|------------------------|----------------------|------------------------|
| Drought stress | 1 | 1250,521** | 235,853** | 2,3408** | 816,750** |
| Salicylic acid | 1 | 368,521** | 157,688** | 60,3008** | 352,083** |
| Bacteria | 3 | 205,243** | 112,979** | 22,7472** | 3,2500 ^{ns} |
| Drought stress × Salicylic acid | 1 | 143,521** | 12,813 ^{ns} | 1,6133** | 18,7500 ^{ns} |
| Drought stress × Bacteria | 3 | 4,0208 ^{ns} | 11,826** | 1,1903** | 3,9167 ^{ns} |
| Salicylic acid × Bacteria | 3 | 0,7986 ^{ns} | 3,7275 ^{ns} | 0,4658 ^{ns} | 1,9167 ^{ns} |
| Drought stress × Salicylic acid × Bacteria | 3 | 0,4653 ^{ns} | 0,1456 ^{ns} | 0,1206 ^{ns} | 0,5833 ^{ns} |
| Error | 32 | 6,3542 | 3,6204 | 0,3775 | 24,2292 |
| CV (%) | | 5,92 | 7,98 | 6,92 | 6,54 |

Table 2. Continued analysis of variance studied traits

| S.O.V | df | Chlorophyll a | Chlorophyll b | Total chlorophyll | a/b ratio |
|--|----|-----------------------|----------------------|-----------------------|----------------------|
| Drought stress | 1 | 379,688** | 184,083** | 1092,521** | 0,1149** |
| Salicylic acid | 1 | 99,188** | 75,000** | 346,688** | 0,0860** |
| Bacteria | 3 | 24,0208** | 17,3889** | 80,4097** | 0,0257 ^{ns} |
| Drought stress × Salicylic acid | 1 | 22,6875 ^{ns} | 24,0833** | 93,5208** | 0,0421 ^{ns} |
| Drought stress × Bacteria | 3 | 8,0208 ^{ns} | 3,0278 ^{ns} | 12,0764 ^{ns} | 0,0363 ^{ns} |
| Salicylic acid × Bacteria | 3 | 6,1875 ^{ns} | 1,0556 ^{ns} | 4,5764 ^{ns} | 3,335 ^{ns} |
| Drought stress × Salicylic acid × Bacteria | 3 | 0,6875 ^{ns} | 0,6944 ^{ns} | 2,0764 ^{ns} | 0,0080 ^{ns} |
| Error | 32 | 14,8750 | 2,7292 | 26,5208 | 0,0160 |
| CV (%) | | 9,57 | 7,96 | 8,43 | 6,46 |

promoting bacteria was significant on fresh weight and shoot dry weight and essential oil percent and was not significant on the other traits. The interactions of salicylic acid and bacteria and also the triple interaction of the drought stress and

Table 3. Continued analysis of variance studied traits

| S.O.V | df | Essential oil yield (mg) | Essential oil percent (%) |
|--|----|--------------------------|---------------------------|
| Drought stress | 1 | 1229,175** | 0,215606** |
| Salicylic acid | 1 | 2102,777** | 0,026078** |
| Bacteria | 3 | 691,189** | 0,008887** |
| Drought stress × Salicylic acid | 1 | 22,2769 ^{ns} | 0,002366 ^{ns} |
| Drought stress × Bacteria | 3 | 9,6135 ^{ns} | 0,003594** |
| Salicylic acid × Bacteria | 3 | 16,0308 ^{ns} | 0,000223 ^{ns} |
| Drought stress × Salicylic acid × Bacteria | 3 | 9,7452 ^{ns} | 0,001054 ^{ns} |
| Error | 32 | 15,5229 | 0,000944 |
| CV (%) | | 11,47 | 8,02 |

Table 4. Mean comparison of different levels of treatments in studied traits

| | | Plant height | Shoot fresh weight | Shoot dry weight | Relative water content | |
|---------------------------------|-------------------|----------------------|----------------------|-----------------------|------------------------|---------------------|
| Drought stress | Normal irrigation | 47,708 ^a | 26,046 ^a | 9,104 ^a | 79,323 ^a | |
| | Drought stress | 37,500 ^b | 21,613 ^b | 8,663 ^b | 71,083 ^b | |
| Salicylic acid | Control | 39,833 ^b | 22,017 ^b | 7,763 ^b | 72,500 ^b | |
| | Salicylic acid | 45,375 ^a | 25,642 ^a | 10,004 ^a | 77,917 ^a | |
| Bacteria | Control | 37,583 ^d | 20,708 ^c | 7,458 ^c | 74,500 ^a | |
| | Azospirillum, | 47,417 ^a | 28,058 ^a | 10,767 ^a | 75,750 ^a | |
| | Bacillus | 41,500 ^c | 23,225 ^b | 8,608 ^b | 75,333 ^a | |
| | Pseudomonas | 43,917 ^b | 23,325 ^b | 8,700 ^b | 75,250 ^a | |
| Drought stress × Salicylic acid | Normal irrigation | Control | 46,667 ^a | 24,750 ^b | 8,167 ^b | 77,250 ^b |
| | Salicylic acid | 48,750 ^a | 27,342 ^a | 10,042 ^a | 81,417 ^a | |
| Drought stress | Control | 33,000 ^c | 19,283 ^c | 7,358 ^c | 67,750 ^c | |
| | Salicylic acid | 42,000 ^b | 23,942 ^b | 9,967 ^a | 74,417 ^a | |
| Drought stress × Bacteria | Normal irrigation | Control | 41,833 ^c | 23,633 ^{bcd} | 7,933 ^d | 79,167 ^a |
| | Azospirillum | 52,667 ^a | 31,150 ^a | 11,233 ^a | 79,667 ^a | |
| | Pseudomonas | 49,333 ^b | 24,283 ^{bc} | 8,517 ^{cd} | 78,667 ^a | |
| | Bacillus | 47,000 ^b | 25,117 ^b | 8,733 ^c | 79,833 ^a | |
| Drought stress | Control | 33,333 ^c | 17,783 ^c | 6,983 ^c | 69,833 ^b | |
| | Azospirillum | 42,167 ^c | 24,967 ^b | 10,300 ^b | 71,833 ^b | |
| | Pseudomonas | 38,500 ^d | 22,367 ^{cd} | 8,883 ^c | 71,833 ^b | |
| | Bacillus | 36,000 ^{de} | 21,333 ^d | 8,483 ^{cd} | 70,833 ^b | |
| Salicylic acid × Bacteria | Control | Control | 34,833 ^d | 19,633 ^c | 6,583 ^c | 71,333 ^b |
| | Azospirillum | 45,000 ^b | 25,767 ^b | 9,467 ^b | 72,833 ^{ab} | |
| | Pseudomonas | 41,000 ^c | 21,633 ^c | 7,650 ^{cd} | 72,833 ^{ab} | |
| | Bacillus | 38,500 ^c | 21,033 ^c | 7,350 ^d | 73,000 ^{ab} | |
| Salicylic acid | Control | 40,333 ^c | 21,783 ^c | 8,333 ^c | 77,667 ^{ab} | |
| | Azospirillum | 49,833 ^a | 30,350 ^a | 12,067 ^a | 78,667 ^{ab} | |
| | Pseudomonas | 46,833 ^b | 25,017 ^b | 9,750 ^b | 77,667 ^{ab} | |
| | Bacillus | 44,500 ^b | 25,417 ^b | 9,867 ^b | 77,667 ^{ab} | |

salicylic acid and bacteria in all traits were not significant.

Mean comparison (Tables 4 to 6) indicated that drought stress reduced plant height, weight fresh and dry weight, relative water content, chlorophyll a, chlorophyll b and total chlorophyll and increased the ratio of chlorophyll a to b and yield and essential oil percent. Korshidi *et al.* (2000) in their study on Coriander (*Coriandrum sativum* L.) showed the in plants under drought stress, intercellular space and the amount of water in their body reduced in order to enter water from the soil in to the plant with more force, which would reduce the relative water content under drought stress and therefore reduce the growth characteristics of the plant.

Also the results this study showed that using salicylic acid increased plant height, fresh and dry weight, relative water content, chlorophyll a, chlorophyll b, total chlorophyll and yield and essential oil percent and reduced the ratio of chlorophyll a to b. It is reported that salicylic acid increases the cell division of apical meristem of seedlings and thereby improve plant growth (Stevens; 2006). Study the mean comparison of the interaction between drought stress and salicylic acid showed that in drought stress conditions salicylic acid increase (recover the reduction) plant height, shoot fresh and dry weight, relative water content, yield and essential oil percent of the plant. As the results of previous research (Raskin, 1992, and Bezorkova, 2001) referred to protective

Table 5. Continued mean comparison of different levels of treatments in studied traits

| | | Chlorophyll a, | Chlorophyll b | Total chlorophyll | a/b ratio |
|---------------------------------|-------------------|-----------------------|-----------------------|-----------------------|----------------------|
| Drought stress | Normal irrigation | 43,125 ^a | 22,708 ^a | 65,833 ^a | 1,907 ^b |
| | Drought stress | 37,500 ^b | 18,792 ^b | 56,292 ^b | 2,005 ^a |
| Salicylic acid | Control | 38,875 ^b | 19,500 ^b | 58,375 ^b | 1,998 ^a |
| | Salicylic acid | 41,750 ^a | 22,000 ^a | 63,750 ^a | 1,914 ^b |
| Bacteria | Control | 38,500 ^a | 19,000 ^b | 57,500 ^b | 2,025 ^a |
| | Azospirillum, | 41,917 ^a | 21,750 ^a | 63,667 ^a | 1,935 ^a |
| | Bacillus | 40,167 ^a | 21,083 ^a | 61,250 ^{ab} | 1,925 ^a |
| | Pseudomonas | 40,667 ^a | 21,167 ^a | 61,833 ^{ab} | 1,939 ^a |
| Drought stress × Salicylic acid | Normal irrigation | | | | |
| | Control | 41,000 ^b | 20,750 ^b | 61,750 ^b | 1,979 ^a |
| Drought stress | Salicylic acid | 45,250 ^a | 24,667 ^a | 69,917 ^a | 1,835 ^b |
| | Control | 36,750 ^c | 18,250 ^c | 55,000 ^c | 2,018 ^a |
| Drought stress × Bacteria | Salicylic acid | 38,250 ^{bc} | 19,333 ^c | 57,583 ^{bc} | 1,992 ^a |
| | Normal irrigation | | | | |
| Drought stress | Control | 42,500 ^{ab} | 21,000 ^b | 63,500 ^{abc} | 2,020 ^a |
| | Azospirillum | 44,333 ^a | 23,000 ^a | 67,333 ^a | 1,937 ^{ab} |
| | Pseudomonas | 42,833 ^{ab} | 23,333 ^a | 66,167 ^{ab} | 1,846 ^b |
| | Bacillus | 42,833 ^{ab} | 23,500 ^a | 66,333 ^{ab} | 1,825 ^b |
| Drought stress | Control | 34,500 ^d | 17,000 ^d | 51,500 ^c | 2,029 ^a |
| | Azospirillum | 39,500 ^{abc} | 20,500 ^{bc} | 60,000 ^{bcd} | 1,934 ^{ab} |
| | Pseudomonas | 38,500 ^{bcd} | 19,000 ^{bcd} | 57,500 ^{cde} | 2,031 ^a |
| | Bacillus | 37,500 ^{cd} | 18,667 ^{cd} | 56,167 ^{de} | 2,026 ^a |
| Salicylic acid × Bacteria | Control | | | | |
| | Control | 36,000 ^b | 18,000 ^d | 54,000 ^b | 1,977 ^{abc} |
| | Azospirillum | 41,000 ^{ab} | 20,500 ^{bc} | 61,500 ^a | 2,005 ^{abc} |
| | Pseudomonas | 39,500 ^{ab} | 19,500 ^{cd} | 59,000 ^{ab} | 2,032 ^{ab} |
| Salicylic acid | Bacillus | 39,000 ^{ab} | 20,000 ^{cd} | 59,000 ^{ab} | 1,960 ^{abc} |
| | Control | 41,000 ^{ab} | 20,000 ^{cd} | 61,000 ^a | 2,053 ^a |
| | Azospirillum | 42,833 ^a | 23,000 ^a | 65,833 ^a | 1,865 ^{bc} |
| | Pseudomonas | 41,833 ^a | 22,833 ^a | 64,667 ^a | 1,845 ^c |
| | Bacillus | 41,333 ^a | 22,167 ^{ab} | 63,500 ^a | 1,891 ^{abc} |

role of salicylic acid in drought stress conditions. Salicylic acid by reduce the lipid peroxidation and by affect the enzymatic and non-enzymatic defense mechanisms has protected corn plants against oxidative stress (Gunes, 2007 and Popova, 1997). It is reported that salicylic acid enhances anthocyanin and chlorophyll content in *Pyrodella* (Popova, 1997). Salicylic acid regulates physiologic processes and plant growth, commonly by affecting the abscisic acid and ethylene (Qinghua, 2008).

According to the results, inoculation of hyssop seed with PGPR increased plant

height, shoot fresh and dry weight, the increase in *Azospirillum* was greater than *Bacillus* and *Pseudomonas* bacteria. Also, inoculation of seeds with bacteria increased chlorophyll b, total chlorophyll and yield and essential oil percent; also increase the amount of yield and essential oil percent was greater in *Azospirillum* than *Pseudomonas* and *Bacillus* bacteria. Sheikhi *et al.* (2014) reported inoculation of calendula plants seeds with bacterial treatments *Azotobacter* sp. and *Pseudomonas putida* significantly increased plant height. Rajai *et al.* (2007) reported use of *Azotobacter* strain AZT-13

Table 6. Continued mean comparison of different levels of treatments in studied traits

| | | Essential oil yield (mg) | Essential oil percent (%) |
|---------------------------------|-----------------------|--------------------------|---------------------------|
| Drought stress | Normal irrigation | 29,296 ^b | 0,316 ^b |
| | Drought stress | 39,417 ^a | 0,450 ^a |
| Salicylic acid | Control | 27,738 ^b | 0,360 ^b |
| | Salicylic acid | 40,975 ^a | 0,407 ^a |
| Bacteria | Control | 25,942 ^c | 0,350 ^c |
| | <i>Azospirillum</i> , | 44,358 ^a | 0,416 ^a |
| | <i>Bacillus</i> | 32,975 ^b | 0,380 ^b |
| | <i>Pseudomonas</i> | 34,150 ^b | 0,387 ^b |
| Drought stress × Salicylic acid | Normal irrigation | | |
| | Control | 23,358 ^c | 0,286 ^d |
| | Salicylic acid | 35,233 ^b | 0,347 ^c |
| Drought stress | Control | 32,117 ^b | 0,434 ^b |
| | Salicylic acid | 46,717 ^a | 0,467 ^a |
| Drought stress × Bacteria | Normal irrigation | | |
| | Control | 20,377 ^d | 0,259 ^d |
| | <i>Azospirillum</i> | 40,217 ^b | 0,366 ^b |
| | <i>Pseudomonas</i> | 27,917 ^c | 0,323 ^c |
| | <i>Bacillus</i> | 28,317 ^c | 0,318 ^c |
| Drought stress | Control | 31,150 ^c | 0,441 ^a |
| | <i>Azospirillum</i> | 48,500 ^a | 0,467 ^a |
| | <i>Pseudomonas</i> | 40,383 ^b | 0,452 ^a |
| | <i>Bacillus</i> | 37,633 ^b | 0,442 ^a |
| Salicylic acid × Bacteria | Control | | |
| | Control | 20,750 ^e | 0,323 ^e |
| | <i>Azospirillum</i> | 36,383 ^b | 0,399 ^{abc} |
| | <i>Pseudomonas</i> | 27,750 ^{cd} | 0,361 ^{cd} |
| | <i>Bacillus</i> | 26,067 ^d | 0,357 ^{de} |
| Salicylic acid | Control | 31,133 ^c | 0,377 ^{bcd} |
| | <i>Azospirillum</i> | 52,333 ^a | 0,433 ^a |
| | <i>Pseudomonas</i> | 40,550 ^b | 0,413 ^{ab} |
| | <i>Bacillus</i> | 39,883 ^b | 0,404 ^{ab} |

with producing 70 mg/L indole acetic acid (IAA) caused an increase in wheat plant height from 35.5 in control treatment to 42.7 cm. Previous research has shown that bacteria *Bacillus*, *Pseudomonas* and *Rhodococcus* have the ability to produce auxin (Naderi, 2012 and Abbaszadeh *et al.*, 2012). On the other hand (Taiz and Zeiger, 2002) also reported that gibberellins do not work alone and other hormones such as auxin interacts with them in different forms. For example, it is known that in some species auxin, regulates metabolism of GA. Therefore, it is possible that *Bacillus* and *Rhodococcus* inoculation treatment by produce large amounts of auxin can stimulate further GA production, thus increasing production leaves and shoots and consequently increasing the biomass is affected by dual effects of auxin and gibberellic. By study the combined effects of drought stress and bacterial inoculation it is observed that in drought stress conditions, bacteria increase plant height, shoot fresh and dry weight, yield and essential oil percent of the plant. The results of Faraji *et al.* (2008) on apply the bio fertilizer on growth indices and quantity traits of the plant are in accordance with these results. Also, it is in accordance with the results of Visani *et al.* (2012) who reported the effect of bio fertilizers on improve the quantity and quality of basil. Jahanshahi *et al.* (2013) showed that the chlorophyll concentration in Coriander significantly increased by using vermicompost and *Azotobacter* and *Azospirillum* bacteria. The results of Sheikhi *et al.* (2014) also demonstrated that *Bacillus*, *Pseudomonas putida*, *Pseudomonas fluorescens* and *Azotobacter* and *Corynebacterium* treatments significantly increased chlorophyll a in calendula compared to the control. In this regard, Banchio *et al.* (2008) also reported that *Bacillus subtilis* significantly increased biomass essential oil content in marjoram. In the study of mean comparison of the interaction between salicylic acid and inoculation showed that using salicylic acid leads to better performance of bacteria and plant height, shoot fresh and dry weight, chlorophyll a, chlorophyll b and total chlorophyll and essential oil yield were higher in using salicylic acid conditions compared to non-use of salicylic acid. The inoculation of calendula seeds with PGPR showed that *Pseudomonas putida* and *Corynebacterium* treatments significantly increased the content of extracted essential oil compared to control and

other bacterial treatments (Sheikhi-Ghahfarokhi *et al.*, 2014). Jamshidi *et al.* (2012) in explaining the results of improved essential oil content due to using organic materials noted since essential oil are terpenoids compounds that their construction units (isoprenoids) such as isopentenyl pyrophosphate (IPP) and dimethylallyl ATP pyrophosphate (DMAPP) need to NADPH and due to the fact that the presence of elements such as nitrogen and phosphorus to composite recent compounds is essential, hence the use of organic materials could be improved phosphorus and other nutrients such as nitrogen by fennel root, and increased this medicinal plant essential and according to this fact that The most important bacteria for phosphate solubilizing are *Pseudomonas* and *Bacillus* (Han *et al.*, 2006). Khosravi (1998) attributed the benefits of *Azospirillum* to develop the hair roots system in different plants and the results of Yousef *et al.* (2004) showed that in Common sage, *Azospirillum* increased plant height and fresh and dry weight of the plant organs.

CONCLUSION

In the present study it was observed that drought stress reduced plant growth traits including yield. Despite of increasing the essential oil percent and essential oil yield under drought stress conditions, the foliar application of salicylic acid prevents yield reduction in drought stress conditions and on the other hand by increasing the essential oil percent led to a dramatic increase in essential oil yield (46.717 mg) compared to normal irrigation and non foliar application of salicylic acid (23.358 mg). On the other hand, the plant growth promoting rhizobacteria by producing metabolites such as growth regulators or different kinds of vitamins as well as improving access to nutrients, caused directly increase plant growth and development and finally increase essential oil percentage and yield. Because of the relationship between the oil percentage increase in drought conditions, improve plant growth conditions by salicylic acid under drought stress conditions and improve the effect of salicylic acid by PGPR, we observed the remarkable differences between essential oil yield in Non-application salicylic acid and without inoculation (20.750 mg) in contrast to foliar application of salicylic acid and inoculation

especially Azospirillum (52.333 mg). So, in order to increase hyssop essential oil yield, inoculation of seeds with bacteria and foliar application of salicylic acid is recommended.

REFERENCES

- Ebrahimpur, F, Eydi zadeh, Kh. Medicinal plants, Tehran, Payam Noor University Press 2007.
- AghaAlikhani M, Iranpour A, Naghdi Badi H. Changes in Agronomical and Phytochemical Yield of Purple Coneflower (*Echinacea purpurea* (L.) Moench) Under Urea and Three Biofertilizers Application. *Journal of Medicinal Plants.*, 2013; **2**(46) :121-136
- Omidbeigi, R. Evaluate chemical types of the Iranian chamomile and comparison them with the modification types. *Tarbiat Modarres Agricultural Journal.*, 1997; 45- 53 pp.
- Omidbeigi, R. Medicinal plants approaches. Ghods Razavi press 2003.
- Omidbeigi, R. Production and processing medicinal plants, 2007; **2**: Ghods Razavi press.
- Khorshidi Benam M. B, F. Rahimzadeh Khoii, M.J. Mirhadi and G. Nour-Mohamadi. Study of drought stress effects in different growth stages on potato cultivars. *Iranian journal of crop science.*, 2002; **4**(1): 48- 59.
- Faraji mehmani, A. Esmaeelpour, E. Sefidkon, F. Abbaszadeh, B. Khavazi, K. Qanbari, A. effect of inoculated with biofertilizers on growth characteristics and quantity and quality yield Savory (*Satureja hortensis* L.), 2002; **6**(4): 870-879.
- Abbas-zadeh, P., Savaghebi, G.R., Asadi-Rahmani, H., Rejali, F., Farahbakhsh, M. Motesharezadeh, B., and Omidvari, M. The effect of fluorescent pseudomonas on increasing the solubility of zinc compounds and improve its absorption by bean (*Phaseolus vulgaris* L.). *Iranian Journal of Soil Research*, **26**(2): 197-206. [In Farsi]
- Banchio, E.C., Bogino, P., Zygadlo, J., and Giordano, W. Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanum majorana* L. *Journal of Biochemistry Systems and Ecology*, 2008; **36**: 766-771.
- Bezorkova, M., Sakhabutdinova, V. Fatkhutdinova, R, Kyldirova, R. A. Shakirova, I, Sakhabutdinova, F. A.R. The role of hormonal changes in protective action of salicylic acid on growth of wheat seedling under water deficit. *Agrochemiya (Russ)*, 2001; **2**: 51-54.
- El Tayeb, M.A. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Grow. Reg.*, 2005; **45**: 215-224.
- Fallahi, J. 2009. Effects of biofertilizers and chemical fertilizers on quantity and quality characterize of Chamomile (*Matricaria chamomilla* L.) as a medicinal plant. MSc Thesis in College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. (In Persian with English Summary)
- Gharib, F.A.E. Effect of salicylic acid on the growth, metabolic activities and oil content of basil and marjoram. *Inter. J. Agri. Biol.*, 2007; **9**: 2. 294-301.
- Gunes, A., A. Inal, M. Alpaslan, F. Eraslan and E. G. Bagci. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *Journal of Plant Physiology*, 2007; **164**: 728—736
- Han, H., Supanjan, S., and Lee, K.D. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant Soil Environ*, 2006; **52**(3): 130-136.
- Hassanzadeh, K., Ahmadi, M., and Shaban, M. Effect of pre-treatment of lemon balm (*Melissa officinalis* L.) seeds on seed germination and seedlings growth under salt stress. *Inter. J. Plant, Ani. Environ. Sci.*, 2014; **4**(3): 260-265.
- Humphrey, A.M. Observation on essential oil distillation in the laboratory. In the 23rd Intern. Symposium Essential oils. Books of abstract, Ayr, scaotland, *OPIV-2.*, 1992; **2**: 145-151.
- Jamshidi, A., Gholavand, A., Sefidkon, F., and Mohamadi Gholtape, A. The effect of nutrition systems on quantitative and qualitative characteristics of Fennel (*Foeniculum vulgare* Mill.) under water deficit stress. *Iranian Journal of Medical and Aromatic Plants*, 2012; **28**(2): 309-323. [In Farsi]
- Kang, G. Salicylic acid changes activities of H₂O₂ metabolizing and increase the chilling tolerance of banana seedling. *Envi. Exper. Botany.*, 2003; **50**: 9-15.
- Khalil, M.Y. How-far would Plantago afra L. respond to bio and organic manures amendements. *Research Journal of Biological Sciences*, 2006; **2**(1): 12-21.
- Khosravi, H.). Survey on abundance and distribution of Azetobacter chorocucum in Theran agricultural soils and study of some physiological characteristics. M. Sc. Thesis, Fac. Agric. Theran University, Iran.
- Leithy, S., El-meseiry, T.A., and Abdallah, E.F. Effect of biofertilizers, cell stabilizer and irrigation regime on Rosemary herbage oil yield

- and quality. *Journal of Applied Research*, 2006; **2**: 773-779.
23. Naderi, M.R. The effect of plant growth promoting rhizobacteria on phytoremediation of Lead by sunflower in a Pb-bearing soil for long term. M.Sc. Thesis in Agroecology, Shahrekord University, 2012; 119 pp. [In Farsi]
 24. Omid Baigi, R. Cultivation of Medicinal Plants and Important Notes about Them. Razavi Astan Ghods., 1994; Press. Pp: 20-40.
 25. Pastirova, A., Repack, M., and Eliasora, A. Salicylic acid induces change coumarin metabolites in *Matricaria chamomilla* L. *Plan. Sci.*, 2004; **167**: 4. 824-830.
 26. Popova, L., T. Pancheva and A. Uzunova. Salicylic acid: Properties, Biosynthesis and Physiological role. *Plant Physiology*, 1997; **23**: 85-93.
 27. Qinghua, S. H. and Z. Zhujun. Effect of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. *Environmental and Experimental Botany*, 2008; **63**: 317-326.
 28. Rajaei, S., Raeisi, F., and Alikhani, H.A. Effect of growth promoters potential of native strains of *Azotobacter chroococcum* on growth, yield and nutrient uptake in wheat. *Scientific Journal of Agriculture*, 2007; **30**(4): 33-47. [In Farsi]
 29. Ram, m., R. Singh., A. A. Naqvi., R. S. Lohia., R. P. Bansal., S. Kumar, Effect of salicylic acid on the yield and quality of essential oil in aromatic crops. *J. Med. Aromatic Pl. Sci*, 1997; **19**: 24-27.
 30. Ramezani, M. Identification of phosphate solubilizing *Pseudomonas* sp. of rice rhizosphere based on 16 SrDNA genotyping. *Middle-East J. Sci. Res.*, 2009; **4**: 4. 348-353.
 31. Ramezani, Y., Khavazi, K., Asadi Rahmani, H., and Popov, M. Genetic diversity and efficiency of indole acetic acid production by the isolates of *Pseudomonads* fluorescent from Rhizosphere of rice (*Oryza sativa* L.). *Amer. -Euras. J. Agric. Environ. Sci.*, 2010; **7**: 1. 103-109.
 32. Rashid, M., Khalil, S., Ayub, N., and Latif, F. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. *Pak. J. Biol. Sci.* 2004; **7**: 187-196.
 33. Raskin, I. Role of salicylic acid in plants. *Annu. Rev. Plant Physiology Plant Mol. Biol.* 1992; **43**: 439-463.
 34. Reddy, A. R., Chaitanya, K. V. and Vivekanandan, M. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology*, 2004; **161**(11): 1189-1202.
 35. Seilsepour M., Baniani E., and Kianirad M. Effect of phosphate solubilizing microorganism (PSM) in reducing the rate of phosphate fertilizers application to cotton crop. Proceedings of the 15th International Meeting on Microbial Phosphate Solubilization Salamanca University, 2002; 16-19 July. Salamanca, Spain.
 36. Sheikhi-Ghahfarokhi, F. Effect of seed biopriming by PGPR bacteria on germination indices, growth and yield of *Calendula officinalis* L. M.Sc. Thesis in Seed Science and Technology, Shahrekord University, 2014; 93 pp. [In Farsi]
 37. Stevens, J. and T. Senaranta. Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma): associated changes in gas exchange, water relation and membrane stabilization. *J. Plant Growth Regul.*, 2006; **49**: 77-83.
 38. Taiz, L. and Zeiger, E. *Plant Physiology*. 3rd edn. Sinaure Sunderland, 2002; 690 pp.
 39. Vande Broek, A. Auxins upregulate expression of the indol-3-pyruvate decarboxylase gene in *Azospirillum brasilense*. *Journal of Bacteriology*, 1999; **181**: 1338-1342.
 40. Wani, P.A., and Khan, M.S. *Bacillus* species enhance growth parameters of chickpea (*Cicer arietinum* L.) in chromium stressed soils. *Food and Chemical Toxicology*, 2010; **48**: 3262-3267.
 41. Weisany, W., Rahimzadeh, S., and Sohrabi, Y. Effect of biofertilizers on morphological, physiological characteristic and essential oil content in basil (*Ocimum basilicum* L.). *Iranian Journal of Medicinal and Aromatic Plants*, 2012; **28**(1): 73-87. (In Persian with English Summary)
 42. Yao, L., Wu, Z., Zheng, Y., and Li, C. Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. *Europ. J. Soil Biol.* 2010; **46**: 49-54.
 43. Youssef, A.A., Edris, A.E., and Gomaa, A.M. A comparative Study between some plant growth regulators and certain growth hormones producing microorganisms on growth and essential oil composition of *Salvia officinalis* L. *Plant Annals of Agricultural Science*, 2004; **49**: 299-311.