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Survey of effects of PGPR and salinity on the characteristics of *Nigella* leaves

Arash Rezaei*, Babak Lotfi**, Mohammad Jafari and Sadegh Bahamin*******

**Graduated M.Sc. in Agronomy, Payam Noor University of Tehran, IRAN.*
***Expert of Jahad University, unit of Ilam province, and Lecturer of Islamic Azad University, Dareshahr Branch, IRAN.*
****Former student of Plant Breeding, Karaj Payam Noor University, Karaj, IRAN.*
*****Young researchers and elite group, Ilam Branch, Islamic Azad University, Ilam, IRAN. and Ph.D student Agro-ecology, Ferdowsi University of Mashhad. Mashhad, IRAN.*

(Corresponding author: Sadegh Bahamin)
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ABSTRACT: Nowadays, medicinal herbs have high economic importance, and considering the development of saline lands in the world, using corrective procedures towards less effects of salinity is necessary. Using growth stimulating bacteria is one of the most important methods in this regard. To that aim, a research in the form of a completely randomized design with 3 duplications and 8 treatments was done on Nigella in Ilam in 2012. The treatments included 0 millimolar (mm) Salinity, 100 mm Salinity, *Azetobacter, Pseudomonas*, 100 mm Salinity + *Azetobacter* + *Pseudomonas* and *Azetobacter* + *Pseudomonas*. The results showed that the research treatments had statistically significant effects on leaf area, leaf protein percentage, and chlorophyll index in 1% level, and on leaf dry weight in 5% level. In the 100 mm salinity treatment + *Azetobacter* + *Pseudomonas*, the leaf protein got 12.33%, 36% more than the 100 mm salinity treatment showing a statistically significant difference. Generally speaking, the results showed that either with separate or with simultaneous use of these bacteria in salinity conditions, the salinity effects on the examined factors can be decreased.

Keywords: *Nigella*, Protein, *Pseudomonas*, *Azetobacter*.

INTRODUCTION

Scientific name of *Nigella* is *Nigella sativa* L. it is dicotyledonous, herbaceous and perennial belonging to the family Ranunculaceae (Bassim, 2003), which grows in West Asia and the Mediterranean regions. Seeds of this plant contain 20-27% protein, 7.3-4.9 % ash, 4.33% carbohydrate and 34.5-38.7% extracted fat (Cheik-Roubou *et al*, 2007). It is more than two thousand years that *Nigella* has been used as a medicinal plant (Toncer & Kizil, 2004). Drought stress is one of the most important stresses in many parts of the world, and especially in dry climates that limited the crop yield (Porudad & Beg, 2003). Water stress is the most common type of stress on the plant and often is associated with long-term shortage of moisture or temporary drought on hot days with high radiation or both (McKersie & leshem, 1994) Many reports has been stated that show lack of water from some turn to intensive stress about disturbance of plants physiological processes change in carbohydrates and nitrogen metabolism and change in proteins construction and enzymes' activity (Sing & Patal, 1996) Reduction of moisture cause to reactions, such as protein degradation and accumulation of free amino acids in the cell result in osmotic pressure (Singh and Patal, 2001). Faker Baher *et al* (2001) study effects of water stress on plant height and number branches and showed the highest levels of water stress branch of plant height and number of shoots significantly reduced. Because of low yield of many of cultivation and medicinal plants in rainfed condition, irrigated condition of these plants, with removal of respective preventive by easy achieving to water sources can be increased its yield. The application of different fertilizer sources of nitrogen fertilizer (manure and chemical) and the spray can be a way to increase production in the medical plants.

The growing approach to the use of medicinal and fragrant herbs in the world makes the importance of cultivating and producing such herbs more clearly. At present, the demand for these herbs as usable products in sanitary and medicinal industries is increasing (Fallahi *et al.*, 2009).

Dispersed medicinal herbs are found in vast geographical areas and collecting them is not economical, so utilizing natural habitats will not meet the needs of pharmaceutical industries, and the extensive use of these herbs will undoubtedly lead to their destruction. Thus, knowing the ecological needs of these herbs and focusing on their cultivating in agricultural and greenhouse levels is a necessity.

The growth of medicinal herbs in natural habitat often accompanies by different environmental tensions like dryness tension, salinity, temperature tension (heat and coldness), radiation, etc. The salinity tension is one of the most important factors that restrict producing agricultural products including medicinal herbs in the world. One biochemical change that occurs in environment tensions including the salinity is producing different kinds of active oxygen that can destruct proteins (Ghorbani *et al.*, 2010). The salinity tension like many other non-biological tensions restricts plants growth. Decreasing growth is a mean to survive tension conditions (Khorsandi *et al.*, 2010). With growing salinity levels, *Cuminum cyminum* seeds growth indexes decrease because of harmful effects of salinity like ions toxicity, physiological dryness effect, and accumulation of salts in the plants (Khomari et al., 2007). Kaya *et al.* (2001) also reported the leaf oldness and shrunk leaf area in *Spinacia oleracea* because of salinity tension. Khorsandi *et al.* (2010) quoting other researchers reported decreased height of two plants, *Origanum vulgar* Aureum and *Ocimum basilicum* and decreased amount of *Foeniculum vulgare* Mill essence. *Trachyspermum ammi*, and *Ocimum basilicum* under the conditions of salinity tension.

Although improving on soil through choosing correct methods of irrigation and drainage for modifying the soil salinity is possible, these methods are not usually economical or possible and other strategies are required to be developed and used. One of these strategies is using biological fertilizers. The term "biological fertilizer" isn't only restricted to organic materials made of livestock manure, plants remains, green manure ,and etc ., but it also refers to the bacterial and fungal micro-organisms, especially plant growth stimulating bacteria (PGSB) and the materials obtained from their activity are considered the most important biological fertilizers. This group of bacteria in the Rhizosphere area cause increase in growth and performance of plants through different mechanisms (Vesi, 2003). From among the bacteria, some species of *Azetobacter* and *Pseudomonas* kinds can be mentioned. The *Azetobacter* bacterium has a special potential for stabilizing Nitrogen biologically and in root parts, it can produce and secrete active biological composition, Auxins, Gibberellins, etc. which increases plants growth (Dehghani, Meshkani *et al.*, 2010). The potential of producing different siderophores using *Azetobacter* and increasing absorption capability of Zn, Fe, and MO , and also the ability of these bacteria to increase the solubility of phosphorus in insolvable mineral compositions have been confirmed that is one way of increasing the activity and absorption capability of food elements (Mrkwaky and Mylik, 2001, Narola et al., 2000). The solvent bacteria of phosphate too cause the increase of solubility of the insolvable phosphorus in soil and the plant growth under the condition of shortage of available phosphorus in soil. The reported initial mechanism for solvent bacteria of phosphate is producing organic acids, from which phosphates acid plays the most important role in solubility of available phosphorus in soil. The production of organic acids through phosphate solvent bacteria has been well confirmed. It seems that gluconic acid is the most abundant mineral solvent phosphate. In addition, ceto gluconic acid-2 is another identified organic acid that can dissolve phosphate (Veskoez et al., 2000). Many researchers have shown that biological fertilizers affect the vegetative indices of agricultural and medicinal plants, like germination, seedling establishment, flowering, and qualitative indices (Manoli, 2008). The quality of plant medicinal compositions depends on plants physiological potential as well as plants growth environment (Hekel and Sasteviko, 2006), and in that case feeding the plants is important. Kaleva (2003) reported that in the *Mentha spicata* plant with the use of *Azetobacter* and *Azospirillum* composition, the essence performance got about 125 kilogram per each hectare that amounted to 85% of the performance of the plots in which chemical fertilizers had been used. Khalil (2006) expressed that using biological fertilizers including *Azetobacter* chroococum significantly increased the quantitative and qualitative performance of *Plantago Psyllium* medicinal plant. Tabrizi *et al.* (2008) show that for *Hyssopus officinalis* plant, the use of biological fertilizers like Nitroxin, *Bacillus*, and *Pseudomonas* increased the performance and plant growth indices compared to the control group. Azaz *et al.* (2009), considering biological fertilizers effect on *Foeniculum vulgare* Mill plant, reported that using *Azetobacter* treatment compared to control group increased *Foeniculum vulgare* Mill performance up to 18 percent. They also reported that applying the treatment led to the increase of the essence amount and its elements especially of Anethole.

According to what mentioned, the goal of this project is examining the effect of salinity and growth stimulating bacteria on the important leaf characteristics, especially the amount of protein, as an important growth factor for the medicinal and spice *Nigella* plant.

MATERIALS AND METHODS

This research was done in greenhouse in Ilam University with 8 treatments and 3 replications in the form of a completely randomized design on the medicinal ad spice *Nigella* plant in 2012. The treatments included 0 mm salinity of sodium chloride (control). 100 mm salinity of sodium chloride + *Azetobacter*, 100 mm salinity of sodium chloride + *Pseudomonas*, 100 mm salinity of sodium chloride + *Azetobacter* + *Pseudomonas* and *Pseudomonas* + *Azetobacter*. The seeds impregnation (*Mentha spicata* seeds as local variants) with growth stimulating bacteria including *Azetobacter* (*Azetobacter chroocucum*) and *Pseudomonas* (fluorescents *Pseudomonas*) was done before planting and in shadow conditions. To do this, at first the required seed amounts for each treatment was chosen, and then they were put in individual plastic bags. To facilitate the sticking of the inoculums into the seeds, it was required to drench the seeds surfaces with an inert and sticky material at first. To do this, we dissolved 10 grams of sugar in 100 grams of water well. After ensuring of the seeds drenching with sugar and water solution, the inculum was added to the seeds and mixed well. Then the seeds were put in individual bags (cardboard kind) and left in the same place (shadow) for two hours to dry while planting to prevent treatments from affecting one another, an individual disposable glove was used for each treatment. To produce salinity level, pure sodium chloride and distilled water were used and after that, the expected salinity was produced in vase soil. In this research, vases with the top diameter of 22 cm for planting were used. The optimum plant density per each vase was assumed 10 plants. To do this, at first 20 bushes in each vase (2 neighboring seeds, the space between bushes 4 cm and the cut depth of 1-2 cm) were planted, and having ensured the germination and establishing of the plants, the thinning operation was initiated.

In flowering stage, from each vase, three plants were taken and the expected indices including leaf surface, leaf protein percentage, chlorophyll index, and leaf dry weight were measured. The bushes leaf surfaces were measured using the Leaf Area Meter model (Li-Cor, LI-1300, USA). Using the Chlorophyll Meter model (SPAD 502, Minolta, Japan), the leaf chlorophyll index was read. To calculate leaf protein percentage, Kjeldahl method was used (Bremnner, 1965). The data analysis was performed using the statistical software SAS, and the means were compared using the LSD method at 5 percent significance. To draw the diagrams, Excel software was used.

DISCUSSION AND RESULTS

Variance breakdown results: The results showed the significant effect of the experiment treatments on leaf surface (LA), leaf protein percentage, chrollphyll index ($p<0.01$) and leaf dry weight ($p<0.05$) for *Nigella* (Table 1).

Table 1: The mean amounts of squares resulted from the variance breakdown of the experiment treatments effects on the *Mentha spicata* leaf charachteristics.

Dry weigh	SPAD	Percentage of protein	Leaf Area	D.F	S.O.V
0.5685 *	234.67 **	34.5656 **	345.34**	7	Treatment
0.2312	34.678	1.567	16.764	16	Residual
15.6767	8.456	8.4545	9.755	-	CV (%)

**, *, and ns show 5 percent and 1 percent significant difference and no significant difference respectively.

The most and least leaf area (individual plant) was seen in the mixed treatment *Azetobacter* + *Pseudomonas* and the salinity 100 mm treatments for as much as 32.5 and 19.8 square centimeters respectively (Fig. 1) whose difference was significant statistically. In addition, in 100 mm salinity treatment + *Azetobacter* + *Pseudomonas*, the *Nigella* leaf area got 29.4 square centimeters, i.e. 19 percent more than the 100 mm salinity treatment. As shown in figure 1, in the 100 mm salinity, the separate use of *Azetobacter* and *Pseudomonas* led to larger leaf area in *Nigella* plant, but the difference was not significant statistically, while with the mixed use of the two bacteria, the significant difference was shown from the view of effects on the leaf area compared to 10 mm salinity. Actually, the result showed that the growth stimulating bacteria in the salinity tension conditions led to the increased leaf area. Khorsandi *et al.* (2010) expressed that the increase of the salinity level has caused a significant decrease of the leaf area in *Agastache foeniculum*. The researchers also said that having decreased the leaf area and having had the chlorized phenomenon and leaves loss due to salinity, the index amount of leaf area decreased too. Salinity raises the needed energy for keeping the natural conditions of a cell (through Osmoregulation) and thus less energy remains for providing the plant growth needs.

Therefore, in general plants are weaker in salinity conditions and have smaller leaves compared to normal plants. The more salt concentration in soil or irrigation water, the less significant the plant growth and the less leaf enlargement speed due to the harmful effects of sodium and chlorine (Salami *et al.*, 2005). Khorsandi *et al* (2010) also reported the significant decrease of the plant leaf area of *Ocimum basilicum* due to the salinity tension. Dehghani, Meshkani *et al* (2010) also reported that using the growth stimulating bacteria caused the increase of leaves numbers and thus the increase of the plant leaf area of wild *Spinacia oleracea* and expressed that probably the increase in leaf area had been due to the increase of Nitrogen absorption by the plant.

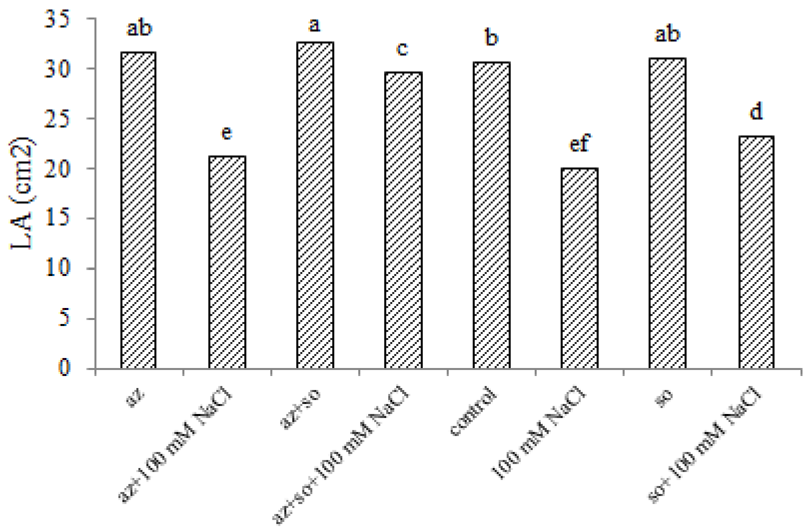

Fig. 1. The effect of the experiment treatments on *Nigella* leaf area.

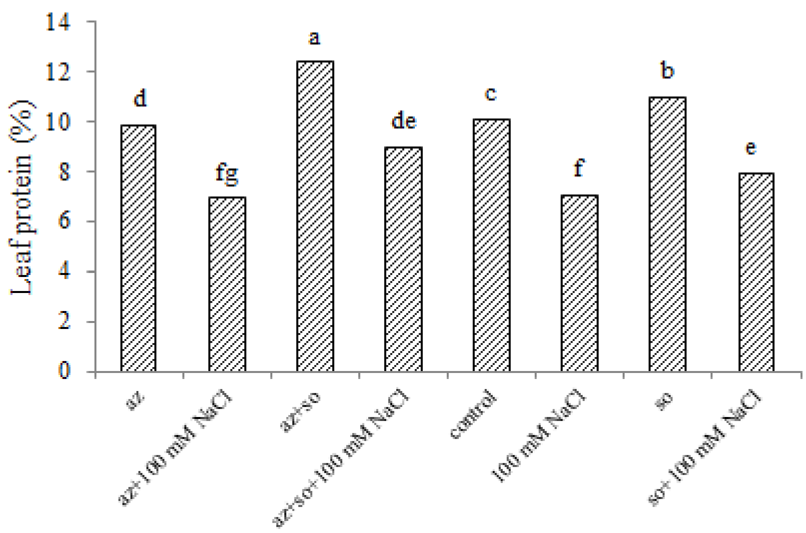

Fig. 2. The effect of experiment treatments on *Nigella* leaf protein.

The amount of leaf protein was also affected by the experiment treatments in a way that the leaf protein in the treatment of mixed inoculation of *Azetobacter* + *Pseudomonas* got 8.9 percent, i.e. 12% more than the way with the lack of seed inoculation with these bacteria (control) (Fig. 2). In the 100 mm salinity treatment + *Azetobacter* + *Pseudomonas*, leaves protein got 12.33 percent, i.e. 36% more than the 100 mm salinity treatment and the difference was significant statistically. One of the bad effects of salinity tension on plants is disrupting their nutrient elements balance and the qualitative change of proteins (Kakero *et al.*, 1993).

One biochemical change happening in the environmental tensions including salinity tension is the production of various kinds of active oxygens that can cause membrane, fats protein and nucleic acids damages (Garratt *et al.*, 2002). Akbari *et al.* (2011) expressed that the bacterial inoculation caused the increase of sunflower seed protein percentage through the increase of growth efficiency due to the production of phyto-estrogens hormone. Han *et al.* reported that the phosphate dissolving bacteria caused the increased of Nitrogen element absorption in *Cucumis sativus* and Capsicum plants in which Nitrogen is the main element forming the protein structure. They also reported that using the phosphate dissolving bacteria from *Bacillus* kind caused the increase of total amount of the protein in pumpkin textures.

The largest amount of leaf dry weight was 0.75 gram for Az + So treatment i.e. 10% more than the amount with the lack of the seeds inoculation (control) (Fig. 3). In 100 mm + *Azetobacter* + *Pseudomonas*, the leaf dry weight got 0.68 gram that was 20% more than the 100 mm salinity treatment; however, this difference was not significant statistically. In addition, the individual use of the growth stimulating bacteria in the salinity tension conditions led to the increase of leaf dry weight. The decrease of leaf growth is the first reaction of glycophyte plants against salinity (Munns & Termaat, 1986). The decrease may be due to the result of the direct effect of salt on the speed of meiosis or the decrease of the duration of cells expansion. Furthermore, it seems that in thses plants, the leaves inability for using the salt transferred from roots in a speed in proportion to its receipt can cause the slow rate of leaf growth and ultimately its death, and with the decrease of leaf area, the plant photosynthetic capacity and the leaf weight decreases correspondingly (Volkmar, *et al.*, 1998). Mahfooz and Sharafoddin (2002) considering the effect of biologic *Azetobacter*, *Pseudomonas*, and *Azospirillum* and their mixes on *Foeniculum vulgare* Mill plant expressed that applying these treatments caused the significant increase of dry weight in the plant textures. The reason has been said to be the phosphate dissolving bacteria ability for making the non-dissolving phosphate in soil useable that normally cannot be absorbed by the plant. Rati et al. (2001) in their research on *Cimbopogon eitratus* observed that the multi-purpose application of the phosphate dissoliving bacteria increased the biological performance of the plant compared to the control group. The use of a phosphate dissolving microorgamism in a bed containing perlit and vermiculite also caused the significant improvement of dry material in *Mentha spicata* herb organs (Kabelo *et al.*, 2005).

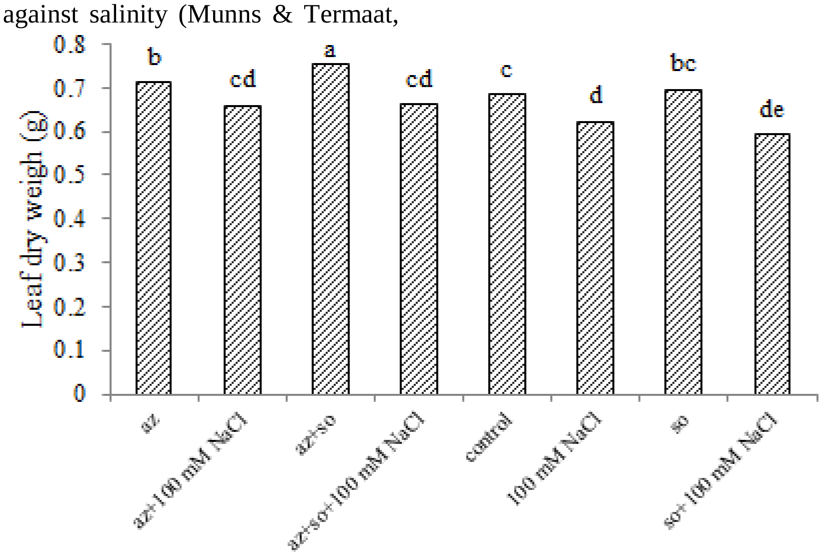

Fig. 3. The effect of the experiment treatments on leaf dry weight (grams) in Mentha.

The highest index for the leaf chlorophyll in the mixed inoculation treatment with *Azetobacter* + *Pseudomonas* got 47.6 that were 30% more than the status of lack of seeds inoculation (control) Fig. 4. In 100 mm salinity treatment + *Azetobacter* + *Pseudomonas*, the chlorophyll index got 30, 32% more than the 100 mm salinity treatment, having a significant difference statistically. In addition, the individual use of *Pseudomonas* and *Azetobacter* in the salinity tension conditions raised the leaf chlorophyll index compared to the 100 mm salinity treatment of Sodium Chloride. Chlorophylls are among the macromolecules, which are damaged in the tension conditions.

The salinity condition causes the lack of Photosynthesis electron transfer, the decrease of opeing guide and the increase of producing various kinds of active oxygens that causes oxidation harms to the photosystems (Maner, 2002, Tabatabaei 2006). Kaya *et al.* (2010) reported the decrease of chlorophyll content in *Spinacia oleracea* leaf under the condition of salinity treatment. Chlorophyll and Nitrogen have a close relation with each other in plants (Eskalmer *et al.,* 2005) and considering the special *Azetobacter* ability for increasing the Nitrogen absorption by the plant, it can be said that *Azetobacter* makes the *Mentha spicata* leaf chlorophyll index increase. Abdolhadi *et al.* (2009) in their research on the evaluation of some *Mentha spicata* varients rection against the growth stimulating bacteria, found out that the inoculation of all the experimental variants by a mix of *Azetobacter* and *Azospirillum* caused the increase of the experimental plants chlorophyll amount.

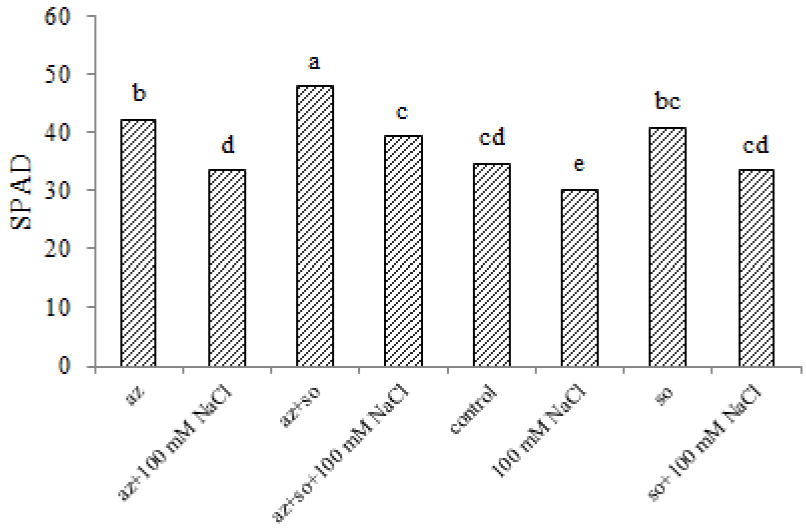

Fig. 4. The effect of experiment treatments on the chlorophyll index of *Nigella.*

Considering the results from the experiment, we can say that using the growth stimulating bacteria including *Azetobacter* and *Pseudomonas*, has made the harmful effects of salinity on the examined indices decrease, leading to the increase of leaf area, protein, dry weight, and chlorophyll index as the important growth indices in the *Nigella* herb. In all the examined characteristics and in the salinity tension treatment, the greatest amount for each characteristic was in the status of mixed use of *Pseudomonas* and *Azetobacter.*

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