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Effect of ascorbic acid in combination with bio and mineral fertilizers on physiochemical properties of roselle (*Hibiscus sabdariffa*, L.) plants grown in different types of soil

Abstract

The impact of bio and mineral fertilizers in combined with ascorbic acid on phytochemical property(N, P and K percentage in roselle herb and its uptake, photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids), anthocyanin pigment and pH value) of roselle plants (*Hibiscus sabdariffa*, L.) under different soils were determined. The data obtained showed that, bio and mineral (NPK) fertilizers in combined with ascorbic acid increased the above phytochemical property of roselle plants under different soils of experiment. The maximum increase of these characters was obtained by the treatment clay soil × 100% NPK + biofertilizers × 400 ppm ascorbic acid, followed by clay soil × 50% NPK + biofertilizers × 400 ppm ascorbic acid as compared to saline loamy sand soil × non fertilizer × zero ascorbic acid treatment, although, the differences between these treatments and mineral fertilizer at the rate of 100% NPK alone were insignificantly. Therefore, it is economically and environmentally recommended to inoculate roselle seeds with mixture of Azotobacter + Bacillus and fertilize these inoculated plants with 50% plus 400 ppm ascorbic acid improve the phytochemical property under clay soil with spraying 400 ppm of ascorbic acid were obtained the best results of this work study.

Keywords: roselle, *Hibiscus sabdariffa* L., nitrogen, phosphorus, potassium, biofertilization, ascorbic acid, soil type, chemical composition

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Introduction

Roselle (*Hibiscus subdariffa* L.), is a shrub belonging to the family Malvaceae. It is thought to be a native to Asia (India to Malaysia) or Tropical Africa. Roselle, in English speaking regions it is called as Rozelle, Sorrel, Red sorrel, Jamanica sorrel, in Egypt it is called "Karkade".[1] There are more than 300 species of hibiscus around the world; one of them is Roselle.[2] Nutrient deficiency in soils is one of the major issues for agricultural production around the globe affecting the quantity and quality of crops.[3] For the improvement of crop yields, farmers have commonly used chemical fertilizers for agricultural production causing an adverse effect on autochthonous organisms and deteriorating the quality of agro-ecosystems and aquatic resources.[4] Nitrogen is required in large quantities for plants growth, since it is the basic constituent of proteins and nucleic acids.[5] Most of the phosphate fertilizers remain unused and accumulate in the soil as precipitates. Apart from this, the accumulated phosphates (in soil) may also reach water bodies causing eutrophication. Therefore, the use of phosphate solubilizing microbes along with the applied phosphate can provide a solution to the menace of accumulation of phosphates in the fields and water bodies. *Bacillus* sp. are well-known rhizobacteria which facilitate the plant growth either by solubilizing of minerals like phosphorus or production of metabolites such as siderophores and phytohormones, and are excellent colonizers of the roots.[6] Bio-as a microbial-based fertilizer composed of different microorganism that has the ability to fix atmospheric N making it available for plant growth which can drastically reduce N- fertilizer input and enhance the yield of wheat. Other results show that Bio-N inoculation, in the presence of 50% the recommended mineral fertilizer, could parallel the yield of fully fertilized corn.[7] Several researchers reported that inoculation of some plants with bio fertilizers (singly or in different combinations with mineral fertilizers) improved plant growth, yield and chemical composition.[8–10] Moreover, several researchers justified the idea that nutrients like N may be taken up through roots and leaves and may spread within the plant.[9,11,12]Also, several researchers justified the idea that nutrients like phosphorus may be taken up through roots and leaves and may spread within the plant.[13–16] Many investigators agreed that application of phosphate dissolving bacteria; *Bacillus megaterium* increased growth characters and chemical composition.[4,10,17,18] Moreover, some works have studied the effect of applying of compounds like antioxidants (like ascorbic acid etc..) on plants[19–22] and agreed that ascorbic acid increased growth characters and chemical composition.

The aim of this work was to study the integrated effect of foliar application with antioxidant ascorbic acid in combos with N&P bio fertilizers plus N,P&K as soil application at different rates on chemical composition of roselle plants.

Material and methods

A field experiment were conducted during the two successive seasons of "2007" and "2008" to study the effect of foliar application with antioxidant ascorbic acid in combos with N&P bio fertilizers plus N&P as soil application at different rates on chemical composition of roselle plants at El-Fayoum governorate conditions. Three types of soil in two experimental stations of the Faculty of Agriculture, namely clay soil at the Dar El-Ramad farm, a saline loamy sand and sandy loam soil at Demo farm. Some chemical and physical characteristics of the experimental stations soils are presented in Table 1.

Biofertilizers namely Azotobacterine (*Azotobacter chroococcum)* as nitrogen fixing bacteria and phosphorein (*Bacillus polymyxa*) as a phosphate dissolving bacteria were obtained from Agricultural Research Center, Ministry of Agriculture, Egypt.The seed of roselle cv. Sabahia 17 were obtained from the Research Center of Medicinal and Aromatic Plants, Ministry of Agriculture, Egypt. Seeds were immersed in Arabic gum solution (16%) as a sticking agent, then, the seeds were mixed with the powder of mixed biofertilizers; inoculated seeds were allowed to dry before sowing, according to Allen (1971). Application of N, P and K fertilizers were 100, 50 and 25% for each fertilizer from that recommended doses by the Ministry of Agriculture, 500 kg ammonium sulfate (20.6% N), 150 kg calcium superphosphate (15% P_2O_5) and 50 kg potassium sulfate/fed (48% K_2O). Nitrogen fertilizer was applied in two equal doses, the first dose during sowing and before the first irrigation, and the second dose after one month from sowing. Phosphorus and potassium fertilizers were applied during soil preparation (Table 2).

Ascorbic acid

Ascorbic acid at rates of zero and 400 ppm were sprayed on roselle plants at the two times, 60 and 90 days from the sowing. Some drops of liquid soap were added to ascorbic acid solution as wetting agent. Plants were sprayed in the early morning with the ascorbic acid solution (300 liter per feddan) from the leaves. The untreated plants were sprayed with the same volume of tap water.

Treatments

The experiment included thirty six treatments with three replicates (three different actual soils × two levels of ascorbic acid × six levels of NPK fertilizers).

The treatments were as following:-

1- Non fertilizer × Zero ascorbic acid.

2- Non fertilizer × 400 ppm ascorbic acid.

3- Biofertilizers × Zero ascorbic acid.

4- Biofertilizers × 400 ppm ascorbic acid.

5- 100% NPK × Zero ascorbic acid.

6- 100% NPK × 400 ppm ascorbic acid.

7- (Biofertilizers + 100% NPK) × Zero ascorbic acid.

8- (Biofertilizers + 100% NPK) × 400 ppm ascorbic acid.

9- (Biofertilizers + 50% NPK) × Zero ascorbic acid.

10- (Biofertilizers + 50% NPK) × 400 ppm ascorbic acid.

11- (Biofertilizers + 25% NPK) × Zero ascorbic acid.

12- (Biofertilizers + 25% NPK) × 400 ppm ascorbic acid.

These treatments were repeated with each one of soil type. Soils were in the main plots, ascorbic acid treatments in the sub-plots and fertilization treatments in the sub-sub-plots. This design offers a good opportunity for obtaining an accurate idea about the optimum level for any main factor as each was applied in the different treatments. Also, the first and second order interactions could be determined.

The experimental field was plowed, rolled and divided into 54 plots, each one of 10.5m^2 area (1/400 fed) and contains 5 rows (3 m width and 3.5 m in length). The seeds inoculated were sown on 1st and 2nd May, in the two experimental seasons. Four seeds were sown per hill (50 cm apart) on one side of the ridge. After one month from sowing the seedlings were thinned to one plant per hill. In this regard, each experimental unit (plot) contains 30 plants (6plants/row) and 12000 plants/fed. All other agronomic practices were followed as recommended in roselle management.

Plant samples

Samples of roselle plants (9 plants) were randomly chosen from each treatment (3 plants for each replicate) in the early morning after 140 days from sowing immediately transferred to the laboratory to study the following plant analysis.

Plant analysis

At the age of 140 days from sowing samples were taken and ground plant materials were wet digested using the H_2SO_4+H_2O_2 mixture as described by Parkinson and Allen[23,24] to determine phosphorus and potassium. The digestion solution was prepared by mixing 350 ml H_2O_2 with 0.42 g Se powder and 14 g $Li_2SO_4.H_2O$ in a flat bottomed boiling flask of 1 liter capacity. Four hundred and twenty ml H_2SO_4 (S.G. 1.84) were carefully added with swirling and cooling. The mixture was then stored at 1°C. Equal portions of 0.2 g of dry ground plant materials were weighted into a 50 ml round bottom long neck reflex flask and digestion, the solution was allowed to cool, diluted to 50 ml, filtered and stored for analysis. The digestions of all samples were analyzed using the following procedures.

1. Phosphorus content in plant materials was determined by ammonium molypdate, hydroquinon and sodium sulfide and determined using colorimetric method according to Jackson.[25]
2. Potassium was determined by flame photometrically according to Page et al.[26]
3. Total nitrogen of plant materials was estimated colorimetrically by using the Orange G dye method as described by Hafez and Mikkelsen.[27]
4. pH determinations in sepals extract were prepared by boiling samples (3 g dry sepals/L) in distilled water for a period of 10 minutes. The pH was measured using pH meter.[28]
5. Anthocyanin was determined according to the method described by Fahmy.[29]
6. At age of 140 days flowering stage (in both seasons) samples of fresh leaves were taken for determination of photosynthetic pigments. Pigments concentrations of extraction solution were calculated according to.[30]

Statistical analysis

A split-split plot design was used. Also, all the collected data were reduced to proper format and units and were statistically analyzed using the procedures outlined by Snedecor and Cochran.[31]

Results

Effect of bio and mineral fertilization as well as ascorbic acid and their interactions under different soil types on chemical composition

1-Nitrogen, phosphores and potasium percentage in roselle herb

Data presented in Table 3–Table 5 indicated the effect of different soil types on N, P&K percentage in roselle herb. Data revealed that soil type had significant effect on N, P&K percentage in roselle herb in the first season but was found a significant increase in the second one. Clay soil gave higher N, P&K percentage in roselle herb compared to sandy loam and saline loamy sand soil in the second season. A significant increment of N, P&K percentage in roselle herb was obtained by mineral NPK in the first and second seasons compared to non-fertilized plants. In regard with biofertilization, data shown in Table 3–Table 5 indicated that application of biofertilization led to significant increase in N, P&K percentage in roselle herb compared to non-fertilizer treatment in the two experimental seasons. The combination between mineral NPK and biofertilizers significantly affected N, P&K percentage in roselle herb in the two experimental seasons. All plants which received 100% NPK and inoculated with bacteria strains were higher than those which not received neither NPK nor biofertilization treatments alone. The most effective interaction treatments were 100% NPK + biofertilization followed by 50% NPK + bio fertilization and then 25% NPK + biofertilization. Data in Tables Table 3–Table 5 show the effect of ascorbic acid at the concentrations (400 ppm) on of N, P&K percentage in roselle herb. Treated plants with 400 ppm ascorbic acid slightly increased of N, P&K % in the first season, while it was a significant in the second one. The interaction between soil type and fertilizer treatments increased N, P&K percentage in roselle herb above the lowest values at the treatment (saline loamy sand soil × non fertilizer).The interaction between fertilizer treatment and ascorbic acid had a positive effect on of N, P&K percentage in roselle herb in the first and second seasons. Concerning, the interaction effect between soil type × ascorbic acid × fertilization treatments (S×AxF), data in Table 3–Table 5 show that of N, P&K percentage in roselle herb was significantly increased by all interaction treatments in the both experimental seasons. Greatly increase of N, P&K percentage in roselle herb was observed, especially in the treatment (clay soil × 400 ppm ascorbic acid × Bio + 100% NPK)

2-Nitrogen, phosphores and potasium uptake (kg/fed)

A significant increment of N,P&K uptake kg/fed by using mineral NPK fertilization was observed in the first and second seasons compared to unfertilized plants. Treating roselle plants with biofertilization, data shown in Table 6–Table 8 indicated that N, P&K uptake was significantly increased compared to non-fertilizer treatment in the two experimental seasons. The response of N, P&K uptake/ fed to combinations of mineral NPK and bio fertilizers were observed in the two experimental seasons. The most effective interaction treatments were 100% NPK + biofertilization followed by 50% NPK + biofertilization and then 25% NPK + biofertilization. The interaction between fertilizer treatment and ascorbic acid had a positive effect on of N, P&K uptake/ fed in roselle herb in the first and second seasons. Data in Table 6–Table 8 show that effect of ascorbic acid at rate of 400 ppm on N, P&K uptake kg/fed was slight increased by 400 ppm ascorbic acid in the first season, while greatly increase was observed on N, P&K uptake kg/fed in the second season. Concerning, the interaction effect between soil type × ascorbic acid × fertilization treatments, data in Table 6–Table 8 show that of N, P&K uptake in roselle herb was significantly increased by all interaction treatments in the both experimental seasons. Greatly increase of N, P&K uptake in roselle herb was observed, especially in the treatment (clay soil × 400 ppm ascorbic acid × Bio + 100% NPK).

3-Photosynthetic pigments content

Data presented in Table 9–Table 11 show that the effect of soil type on photosynthetic pigments content. Data revealed that soil type has no significant effect on photosynthetic pigments content except, chlorophyll b in the first season, but has a significantly increase in the second season. The clay soil gave the highest values of chlorophyll a, chlorophyll b, and carotenoids, compared to sandy loam and saline loamy sand soil in the second one. Data in Table 9–Table 11 indicated that clay soil increased photosynthetic pigments content as compared to sandy loam or saline loamy sand soil. The photosynthetic pigments i.e. chlorophyll a, b and carotenoids of the roselle fresh leaves were significantly responded to mineral NPK fertilization in comparison to check treatment (non fertilizer) in the two growing seasons as shown in Table 9–Table 11 Also, data listed in Table 9–Table 11 revealed that biofertilization significantly promoted photosynthetic pigments content in comparison with non-fertilized plants. Inoculating seed plants with biofertilizer insignificant increased chlorophyll a, b and carotenoids in the first season, while significantly increase was observed in the second season. The interaction between soil type and fertilizer treatments caused an increase of pigments content especially, at the treatment clay soil × Bio + 100% NPK above the lowest values at the treatment saline loamy sand × non fertilizer. Moreover, the data in Table 9–Table 11 reported that treated plants with ascorbic acid at rate of 400 ppm caused greatly an increase of photosynthetic pigments content except chlorophyll b in the first season.The interaction between soil type × ascorbic acid × fertilization treatments were showed in Tables (9,10 and 11). The chlorophyll a, b and carotenoids were significantly increased by all interaction treatments in experimental of both seasons.

4-Anthocyanin content

Data presented in Table 12 show that clay soil gave the highest anthocyanin content in the sepals of rosel1e plants compared to sandy loam soil or saline loamy sand soil in the first season and second season. Supplement of 100% NPK fertilization treatment significantly increased anthocyanin pigment content in the sepals of rosel1e plants in comparison to the untreated plants Table 12. Also the data indicated that treated roselle plants with bio fertilization increased anthocyanin pigment content compared to non fertilized plants in the experiment two seasons. Data tabulated in Table 12 show that the anthocyanin pigment content in the sepals of rosel1e plants was significantly increased by applying rate of 400 ppm ascorbic acid compared to unsprayed plants in the first and second seasons, respectively.

The interaction effect between soil type and fertilizer treatments slightly increased anthocyanin content in the first season, while, the increase was observed by the treatment (clay soil × Bio + 100% NPK) above the lowest value for the treatment (saline loamy sand soil × non fertilizer) in the second season Table 12. Also, the interaction effect between (clay soil × 400 ppm ascorbic acid) was also increased anthocyanin content. The combination effects between soil type × ascorbic acid × fertilization treatments are showed in Table 12. Data cleared that anthocyanins content was a significantly increased by all interaction treatments in the experiments of both seasons.

5-pH values

Data presented in Table 13 show the effect of different soil types on pH values in the sepals of roselle plants. Data revealed that clay soil gave the higher pH value compared to sandy loam or saline loamy sand soil in the first season only. Also the data recorded in Table 13 show that pH values in sepals of roselle were significantly increased (that mains decrease of the acidity) by applying the treatment 100% NPK in comparison with non fertilized plants. Biofertilization treatment led to an increase in pH value compared to untreated plants Table 13. In respect with the combined effect of mineral and biofertilization treatments, it could be noticed that combination treatments, in most cases, affected pH values. The most effective interaction treatments

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were 100% NPK + biofertilization followed by 50% NPK + biofertilization than 25% NPK + biofertilization. The interaction effect between clay soil × Bio + 100% NPK increased pH values above the lowest values (at the treatment saline loamy sand soil × non fertilizer of the first and second seasons, respectively. Regarding pH value, data in Table 13 show that treated roselle plants by 400 ppm ascorbic acid greatly increased pH values compared to non sprayed plants in the first and second seasons. In respect with the combined effect of mineral and biofertilization treatments, it could be noticed that combination treatments, in most cases, affected pH values. The most effective interaction treatments were 100% NPK + biofertilization followed by 50% NPK + biofertilization than 25% NPK + biofertilization. The interaction effect between fertilizer and ascorbic acid treatments was increased pH values at the treatment (Bio + 100% NPK × 400 ppm ascorbic acid), while the lowest values at the treatment (non fertilizer × zero ascorbic acid) in the first and second seasons, respectively. Regarding, the interaction effect between soil type × ascorbic acid × fertilization treatments, data in Table 13 revealed that the pH values were significantly increased by all interactions among treatments in the experiments of both seasons. These interactions increased pH in the first and second season, respectively Table 14- Table 16.

Discussion

From the aforementioned discussion of the results regarding the influences of mineral nitrogen, phosphorus and potassium fertilization and inoculation with N-fixing bacteria strains (*Azotobacter chroococcum*) and (*Bacillus polymyxa*) as phosphate dissolving bacteria, it could be concluded that the beneficial and unique roles of NPK fertilization and N and P biofertilization were responsible for stimulation of different physiological and biological processes which reflected on enhancing different chemical constituents of roselle plants. Biofertilizers, which can be defined as preparation containing, live cell of efficient strains of nitrogen fixing organisms or phosphate-solublizing bacteria could be used instead of chemical fertilizers. Moreover, these components increase the availability of nutrients in a form, which can be easily assimilated by plants.[32] Gaudry[33] (mentioned that bacteria of Azotobacter could produce Indol-acetic acid and cytokinin, which increase the surface area per unit root of nutrients from soil. The increase of macronutrients i.e. N, P and K in herb of roselle plants may be due to the vigorous vegetative growth and root system area, consequently, increasing nutrients uptake by plant. The positive effect of clay soil on nutrients uptake/plant may be due to its mineral enrichment and higher content of organic materials than other soils. While sandy soil doesn't hold many nutrients and less organic matter. In this respect, Moawad[34] found that clay soil is usually mineral rich and high fertility. However, they have high content of organic matter than saline loamy sand. The chemical compositions were increased in clay soil than saline loamy sand; this might be due to high content of organic matter and availability nutrients. On the other hand, the increase in salt concentration led to an unfavorable effect on nutrient balance.

Nitrogen fixation can be defined as the phenomenon of conversion of free nitrogen into nitrogenous salts to make it available for absorption by plants. The fixation of nitrogen can be either a physical nitrogen fixation or a biological nitrogen fixation.[35] N-fixing bacteria strains produce some amino acids, namely, aspartate, glutamate, serine and glutamate,[36] improve water status, augmenting the activity of nitrate reductase, produce siderophores which are responsible for improving iron nutrition of the plant.[37] Plant inoculation with nitrogen-fixer such as Azotobacter affected many foliage parameters. These changes were directly attributed to positive bacterial effects on mineral uptake by the plants, enhancement in uptake of NO_3^-, NH_4^+, PO_4^{--}, K^+ and Fe^{++} by nitrogen fixing bacteria was proposed to cause an increase in foliar dry matter.[38] Effect of biofertilizer on chemical constituents of leaves had been reported by several researchers[39] such beneficial effects of the non- symbiotic N_2-fixing acteria on the morphology and/or physiology of root system which; consequently, promoted uptakes of mineral nutrients nitrogen, phosphorus and potassium. Moreover, phosphate dissolving bacteria leads to an increment of available phosphorus in plant tissue, which affects a large number of enzymatic reactions that depend on phosphorylation.[40] Moreover, inoculating the plants with or without mineral phosphorus fertilizers augments the concentration of phosphorus in the soil and plant tissue and improves the uptake of minerals and water use efficiency.[41]

Recently, this is a widespread use of natural and safety substances such as antioxidants, particularly ascorbic acid, for enhancing the health, growth and productivity of many crops. Since, antioxidants have synergistic effect on some chemical constituents under favorable and unfavorable environmental condition, due to these compounds as non enzymatic material and have a beneficial effect on catching the free radical or the active oxygen species namely single oxygen, super oxide, hydrogen peroxide, hydroxyl radicals and ozone that producing during photosynthesis and respiration process.[42] Seif El-Yazal[19] that some antioxidants which belongs ascorbic acid may help to overcome some of these inhibitory effects and beneficial effect of antioxidant on some chemical constituents of plants.

The promoting effect of ascorbic acid on leaf pigments concentration might be attributed to their enhancing effect on the nutritional status of roselle plants. In this respect, Farag[43] stated that most antioxidants are responsible for accelerating the biosynthesis of various pigments leading to the increase in biosynthesis of sugars. Ahmed and Abd El-Hameed[44] who reported that the effect of antioxidants on producing healthy plants leads to enhance the plants to have a great ability for elements uptake in leaves N, P, K, Zn, Mn, Fe and Cu. Foyer et al.[45] pointed out that antioxidant prevented enzyme inactivation, the generation of more dangerous radicals and allowed flexibility in the production of photosynthetic assimilatory power. Morever, electron transfer to O_2 prevented over reduction of electron transport chain, which reduced the risk of back reactions within the photosystem. The beneficial effect of antioxidant (ascorbic acid) on growth, yield and chemical compositions as well as improved quality of several plants was reported by several workers such as[45–47] on different ornamental plants including roselle plants.

Finally, from the present results, it could be concluded that the application of bio application of nitrogen and phosphorous plus ascorbic acid greatly increased chemical compositions of roselle plants due to that, these element participate in the different metabolic processes, so that the use of N&P as bio and soil application could be used for producing plants with high sufficient cellular solutes enable them to overcome salinity of soils, and consequently producing greatest yield with high quality of rosella plants.

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Conflicts of interest

Authors declare no conflicto of interest exists.

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