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EFFECT OF MINERAL NITROGEN LEVELS AND N_2-FIXING BACTERIA ON GROWTH, YIELD AND STORABILITY OF GREEN ONION PLANTS DURING COLD STORAGE PERIODS

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ABSTRACT

Two field experiments were carried out during winter seasons of 2012/2013 and 2013/2014 at the Agriculture Research Farm, El-Kassasien Hort. Research Station, Ismalia Governorate, Egypt, and Laboratory of Handling of Vegetable Crops Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt, to investigate the effect of partially substituting of mineral N fertilization with N_2-fixing Azospirillum and Azotobacter spp. on growth, dry weight, root system and yield of green onion (Allium cepa L.) cv. Giza 20 grown under sandy soil conditions using drip irrigation system. It aimed also to study the effect of the abovementioned treatments on green onion plants during cold storage at different periods; i.e., 0, 5, 10, 15 and 20 days.

Fertilization green onion plants with 100% mineral N recorded the highest value of morphological characters, root system, yield and its components, weight loss%, total chlorophyll and leaf extension followed by fertilization with 75%.

Generally, inoculation green onion plants with Azospirillum plus Azotobacter gave the highest value of growth characters, yield and its components as well as quality parameters during cold storage periods. The interaction treatments between 100% mineral N or 75% and inoculation with Azospirillum plus Azotobacter gave the maximum values of growth parameters, yield and its components without significant differences between them.

As to the quality parameters of green onion during cold storage at 0 ^{o}C and 90-95 RH for 20 days, the results showed that weight loss%, leaf extension and curvature score increased as the storage period prolonged, while total chlorophyll and TSS% decreased by prolongation the cold storage periods. The interaction treatments among 100% mineral N or 75% and inoculation with Azospirillum plus Azotobacter and storage green onion plants for 15 days at 0 ^{o}C

and 90-95 RH recorded the beast values of weight loss%, total chlorophyll, TSS% and leaf extension as well as curvature score.

Key words: Onion, mineral nitrogen, *Azospirillum*, storage period, yield, *Azotobacter*

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important crops in Egypt used for local consumption and also as exportation commodity. Onion, compared to other fresh vegetables, in relatively high in food energy and medical components, intermediate in protein and rich in riboflavin and calcium. There are along lest of countries importing onion, which shows that onion is an important item in the world trading. Furthermore, it is the second important horticulture crop after tomatoes (Griffiths *et al.,* 2002).

Using mineral fertilizers (NPK) without rationalization may cause environmental pollution as well as contaminate the underground water. Therefore, there was a great attention to use biofertilizers in the production of onion in order to reduce the contamination of plant and soil with different elements, to reduce the usage of mineral fertilizers, to produce clean crop and also to improve the soil properties. Biofertilizer (microbial inoculation), which contain efficient strains of nitrogen fixing *Azospirillum lipoferum* and *Azotobacter chrococcoum*, could be used partially instead of chemical fertilizers. Moreover, these N_2 fixing bacteria increase the availability of fixing atmospheric nitrogen in form that can be easily assimilated or to make them absorbable by plants (Subba Rao, 1993).

Nitrogen is one of the essential mineral elements for plant growth and one nutrient that is the most frequently in short supply in cultivated soils around the world. The use of biofertilizer such as nitrogen fixing bacteria may reduce the amount of nitrogen application and consequently reduces production cost (Saad *et al.,* 1999). In this respect, Fertilization of garlic plants with N at 100 Kg / fed. significantly increased plant height, leaf number, dry weight of leaves, bulb and total dry weight / plant, N, P and K content in bulb as well as total and exportable yield (Abou El-Magd *et al.,* 1998). Application of high dose of nitrogen (120kg N/fed.) increased plant height, number of leaves /plant, dry weight of different onion plant organs ,total yield and the bulbs weight loss, but it decreased the bulbing ratio (El-Tantawy and El-Beik, 2009) .

In addition, inoculation of 100 Kg garlic cloves with 1 Kg biogen increased number of leaves / plant, total dry weight / plant, total yield, marketable yield and the content of N, P and K in bulb tissues compared with the control (Ali *et al.,* 2001). Using 7 Kg nitrobein (Nr) or applying mineral nitrogen fertilizer at 100 Kg / fed. increased plant height, diameter of both neck and bulb, dry weight of bulb and leaves, total yield and uptake of N, P and K by garlic plant compared with the control (EL-Shabasi *et al.*, 2003). Moreover, fertilizing garlic plants with 100% mineral N combined with 3 Kg Nr/ fed. gave the highest length and recorded maximum values of leaf number, neck and bulb diameter, total dry weight / plant, average bulb weight and total yield (Bardisi *et al.*, 2004 a and b).

Azotobacter + *Azospirillum* combination is the best for onion as compared to others so far as the sustainability in production and environmental consideration are concerned (Ghanti and Sharangi, 2009). Fertilization of garlic plants with 100% mineral N (120kg N/fed.) or 75 % N plus 1kg N_2 – fixers recorded the highest values of growth characters, bulbing ratio and total yield/ fed. (Nour *et al.*, 2010).

Biofertilizers have beneficial return to increase population of soil microorganisms, especially in the surface layer of root rhizosphere, that create substances which stimulate plant growth (Awad, 2002). Further, combination between both mineral and bio-fertilizer is the most imperative factors needed to diminish agricultural chemicals, protect the air, soil and water from pollution as well as acquiring high yield quality. Free-living nitrogen-fixing bacteria; e.g., *Azotobacter chroococcum* and *Azospirillum lipoferum,* were found to have not only the ability to fix N but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid which could stimulate plant growth, absorption of nutrients and photosynthesis (Fayez *et al*., 1985). Many researchers reported that using non-symbiotic N_2 fixing bacteria as Halex-2 (*Azotobacter* + *Azospirillum* + *Clebsiella*) with adding minerals fertilizers led to improve vegetative growth, yield and quality of several economic vegetables like onion (Yaso *et al*., 2007), and garlic (Nour *et al.*, 2010, Abdel-Razzak and El-Sharkawy, 2013). Many researchers reported that, there was a considerable increase in weight loss percentage with the prolongation of cold storage period of several economic vegetables like, Emam (2009) on green onion, and Ismail and Mohamed (2014) on sweet pepper.

Chlorophyll reading in different plant organs decreased gradually with the prolongation of cold storage period, Emam (2009) on green onion and Mohamed (2014) on pea.

Total soluble solids (TSS) was decreased gradually and continuously with the prolongation of cold storage period, Emam (2009) on green onion and Ismail and Mohamed (2014) on sweet pepper.

Continues increases in leaf extension of the green onion cut end were evident where storage period was extended to 16 days (Emam, 2009). Leaf curvature stored to be shown after 8 days of cold storage, extending the period of storage up to 16 days at 0 ^{o}C plus 2 days at 20 ^{o}C resulted in a significant higher leaf curvature score (Emam 2009 on green onion).

Therefore, the aim of the present study was to clarify the effects of partial substitution of chemical N fertilizer by inoculation with *Azospirillum lipoferum* and *Azotobacter chrococcoum* on growth, root system and yield as well as keeping quality of fresh cut green onion (*Allium cepa* L.) grown under sandy soil conditions during cold storage.

MATERIALS AND METHODS

Field experiment:

This experiment was carried out during the winter seasons of 2012/2013 and 2013/2014 at the Agriculture Research Farm, El-Kassasien Hort. Res. Station, Ismalia Governorate, Egypt, to clarify the effects of partial substitution of chemical N fertilizer by inoculation with *Azospirillum lipoferum* and *Azotobacter chrococcoum* on growth, root system and yield of green onion plants (*Allium cepa* L.) under sandy soil conditions. Microorganisms, *Azospirillum lipoferum* and *Azotobacter chrococcoum* were obtained from microbiology department, Soil Water and Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt.

*Inoculum preparation***:**

Azospirillum lipoferum culture was prepared on semi-solid malate medium (Dobereiner, 1978) for 48h at 30 ^{0}C. Whereas, *Azotobacter chrococcoum* culture was prepared on modified Ashby's medium (Abd El-Malak and Ishac, 1968). The mixture of bacterial cultures (1:1) containing (1×10^{7} cells / ml) was injected into sterilized beatmoss (50 ml culture / 250 g) per bag and then each bag was mixed thoroughly to be ready for use as an inoculum for application.

The experimental soil was sandy in texture with 96.5 and 95.6% sand, 1.7 and 1.6 % silt, 1.8 and 2.8% clay, 8.1 and 8.1pH, 0.03 and 0.08 % organic matter, 5.4 and 6.9 ppm N, 5.5 and 6.2 ppm P and 52 and 64 ppm K in the 1st and 2nd seasons, respectively. This experiment included twelve treatments, which were the combinations between three of mineral nitrogen rates and three biofertilizers (N_2 fixing bacteria) treatments in addition to the control. The treatments were arranged in a split plot design with three replicates, mineral nitrogen rates treatments were randomly assigned in the main plots, while biofertilizers treatments were randomly distributed in the sub-plots as follows:

1- **Mineral N fertilizer rates:** 1.1. 100 % of the recommended dose (90 Kg N/fed.),

1.2. 75 % mineral N (67.5Kg N/ fed.) and

1.3. 50% mineral N (45Kg N/ fed.).

2- **Biofertilizers treatments:** (N_2 fixing bacteria).
2.1. Control (without treatment),
2.2. *Azospirillum lipoferum*,
2.3. *Azotobacter chrococcoum* and
2.4. Combination (*Azospirillum lipoferum* + *Azotobacter chrococcoum*).

N_2-fixers inoculum (1 Kg / fed.) was mixed with onion seeds by adding Arabic gum solution, then the seeds coated with the inoculum before sowing, the treated seeds were directly sown in the same day. The source of biofertilizers (Microorganisms), *Azospirillum lipoferum* and *Azotobacter chrococcoum* was microbiology department, Soil Water and Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt. While, onion seeds of Giza 20 cv. were obtained from Field Crops Institute, Agriculture Research Center, Egypt.

Seed sowing was done on September 21st and 26th in 2013 and 2014 seasons, respectively. Treated seeds were sown on two sides of the dripper line in hills at distance of 10 cm apart. At 30 days from sowing, plants were thinned leaving one plant / hill. The experimental unit area was 10.5 m^2 it contained three dripper lines with 5 m in length and 70 cm in width. Mineral nitrogen was applied as ammonium sulfate (20.6 %N), at five equal portions after 30, 45, 60, 75 and 90 days from sowing. All plots received equal amounts of compost at a rate of 30m^3/feddan during soil preparation, the other recommended agricultural practices for commercial onion production were followed.

Data Recorded: The obtained data in this study were as follows:

Morphological Characters:

A random sample of five plants from every experimental unit was taken after 115 days from sowing to investigate the following growth parameters: Plant height (cm), number of leaves /plant, neck diameter (cm), bulb diameter(cm), as well as bulbing ratio according to the equation of Mann (1952).

Bulbing ratio = Neck diameter / Bulb diameter

Dry weight:

The different parts of onion plant; i.e., leaves and bulb were oven dried at 70 ^{0}C till constant weight and then the dry weight of leaves, bulb and total plant dry weight were recorded.

Root system traits:

The root of onion plants were carefully separated by washing the sand from them and roots were placed in a flat glass dish containing a little amount of water. Roots were straighted by forceps, so that they can not overlap and were held in position, according to Helal and Sauerbesk (1986), and the following data were recorded per root: root length (cm), fresh and dry weight of root (g), and root volume (cm^3).

Yield and Its Components:

At harvesting time (about 120 days after sowing) all plants from each plot were harvested to measure plant weight (g), yield/plot (kg), total yield / fad. (kg) and relative yield.

Storage experiment:

This experiment was conducted to study the effect of partial substitution of mineral N fertilizer by the inoculation with *Azospirillum lipoferum* and *Azotobacter chrococcoum* on keeping quality of fresh cut green onion during cold storage. In this experiment, green onion plants of the field experiment were harvested at suitable maturity stage of marketing on January 21st and 27th (120 days from seeds sowing) in the first and second season, respectively, then plants were transferred directly to the laboratory of Post Harvest and Handling of Vegetable Crops Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt, and kept overnight at 0 ^oC and 90-95% relative humidity (RH). Plants were trimmed (leaf tips and root cut) and stored in uniform size (15:25mm bulb diameter and 25cm length). Defect free plants were bunched (10 plants/bunch) and tied using rubber bands. Twelve bunches were prepared for each treatment, placed in carton box (30 X 20 X10cm), then stored at 0 ^oC and 90-95% RH for 20 days. Three replicates of each treatment were randomly taken every five days intervals for determining the post harvest measurements. The experimental design was completely randomized with three replicates. Physical and chemical properties were recorded as follow:

Weight loss percentage: It was estimated according to the following equation:

$$\text{Weight loss (\%)} = \frac{\text{(Initial weight of plants} - \text{Weight of plants at sampling dates)}}{\text{(Initial weight)}} \times 100$$

Photosynthetic pigments: Total chlorophyll was measured in fresh leaves by using Minolta chlorophyll meter SPAD-501as SPAD units.

Total soluble solids (TSS): It was determined by using a hand Refractometer according to the methods mentioned in A.O.A.C. (1990).

Inner leaf extension: It was measured with a vernier caliper, considering the growth of leaves from the 25 cm initial plant length to the upper leaflet and expressed in cm.

Curvature score of 1:5 was used where 1= none, 2 curvature of stem or leaf up to 15^o from the horizontal position, 3, 15:30^o, 4, 30:45^o and 5, $\geq$ 45^o (Hong *et al.,* 2000).

Statistical Analysis:

Data of the field experiment and cold storage experiment were statistically analyzed by using MSTAT statistical software and the treatments means were compared by using LSD at 0.5 level of probability according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Field Experiment

Morphological Characters : Data presented in Table 1 show clearly the effect of mineral nitrogen levels and nitrogen fixing bacteria on morphological characters of green onion plants. It is clear from the data that, morphological characters were increased considerably and consistently with the increasing mineral nitrogen level from 50% up to 100% of the recommended dose, whereas the highest value of morphological characters were recorded by 100% mineral nitrogen, while the lowest value was recorded by 50% mineral nitrogen. The increment in vegetative growth and consequently in dry weight of onion plants due to application of high rate of mineral nitrogen (90 kg N/fed.) may be attribute to the pronounced role of nitrogen in plant metabolism. Nitrogen is a constituent of proteins, enzymes, hormones, vitamins alkaloids, chlorophyll and photosynthesis which led to an increase in plant metabolism and vegetative growth expressed as plant height, number of leaves/plant, as well as dry weight of plant (Reddy and Reddi 2002). The favorable effect of mineral nitrogen fertilizer on morphological characters of green onion plants was in harmony with the results reported by El-Tantawy and El-Beik (2009) on onion and Abou El-Magd *et al.* (1998) on garlic.

Regarding to N_2 fixing bacteria, results in the Table 1 indicate also that inoculation of green onion plants with N_2 fixing bacteria as seed treatment enhanced all studied morphological characters as compared to untreated plants, the highest value of morphological characters was recorded by the combination between *Azospirillum lipoferum* plus *Azotobacter chrococcoum*. Such effect of the above mentioned treatment could be attributed to the activity of bacteria in the absorption zone of plant roots by improving soil fertility and consequently plant development by N_2- fixation and due to releasing of certain other nutrients; i.e., Fe, Zn and Mn (Bhonde *et al*., 1997) through the break down of organic materials in the soil and make these elements in available forms. Similar results were obtained by Yaso *et al*. (2007) on onion, Nour *et al.* (2010), Abdel-Razzak and El-Sharkawy (2013) on garlic.

Concerning to the effect of interaction between the two studied factors results in Table 2 obviously reveled significant effect in both seasons among the various interactions, generally the interaction between 100% nitrogen and inoculation green onion plants with *Azospirillum lipoferum* plus *Azotobacter chrococcoum* significantly increased all the studied morphological characters followed by the interaction between 75% nitrogen and inoculation with the combination between the two N_2 fixing bacteria. These results are in agreement with those of Nour *et al.* (2010) on garlic.

Root system:

Data in Table 3 show the effect of mineral nitrogen levels and seed treatment with N_2 fixing bacteria and their interaction on green onion root system expressed as root length, fresh and dry weight of root per plant as well as root volume. It is obvious from the data that, fertilizing green onion plants with 100% mineral nitrogen significantly increased these characters except root length in 1st season, on the other side the lowest value of root system parameters was recorded from green onion plants which fertilized with 50% mineral nitrogen. These results are true in both seasons of study.

As for the effect of N_2 fixing bacteria; viz., control, *Azospirillum, Azotobacter* and the combination between *Azospirillum* plus *Azotobacter* on root system parameters. The results in Table 3 show that inoculation green onion plants with N_2 fixing bacteria as seed treatment significantly increased root fresh weight and root volume, but it did not record any significant effect on root length and root dry weight. Inoculation of green onion plants with *Azospirillum* plus *Azotobacter* recorded the highest value of root system, while the lowest value was recorded from untreated plants in the two seasons. Regarding to the effect of interaction between mineral nitrogen levels and N_2 fixing bacteria, the results listed in Table 4, clearly show that the interaction between the two studied factors had significant effect on all measured root system parameters expressed as root length, fresh and dry weight as well as root volume. In general, the interaction between 100% mineral nitrogen (90 kg/fed.) and the three treatments of biofertilizers recorded the highest value in this respect, followed by the interaction between 75% mineral nitrogen (67.5 kg/fed.) and inoculation with *Azospirillum* plus *Azotobacter* without significant differences among them on the same characters described above.

Yield and Its Components:

The results in Table 5 show the effect of mineral nitrogen levels and N_2 fixing bacteria on yield and its components of green onion plants; i.e., plant weight, yield per plot, total yield per feddan and relative yield. It is obvious from such data that fertilizing green onion plants with 100 % mineral

nitrogen (90kg N/fed.) significantly increased yield and its components as well as relative yield. The increment was considerable and consistently with the increasing nitrogen level from 50% up to 100%. The increment in total yields due to application of 100 %N (90 kg N /fed.) could be attributed to the increment of vegetative growth and rising photosynthesis production which associated with increment in bulb size and single bulb weight as recorded by Khan *et al.* (2002). Similar results were obtained by El-Tantawy and El-Beik (2009) on onion and Abou El-Magd *et al.* (1998) on garlic. Concerning to N_2 fixing bacteria, the results in the same Table show that inoculation of green onion plants with N_2 fixing bacteria significantly increased yield and its components. Treating green onion plants with the combination between *Azospirillum* plus *Azotobacter* recorded the highest value of yield and its components expressed as plant weight, yield per plot and total yield per feddan. On the other hand, the lowest value in this respect was recorded from untreated plants. The increases in total yield of green onion plants were about 23.4 and 21.1 for the combination treatment, 11.9 and 10.1 for *Azospirillum* and 13.5 and 7.1 for *Azotobacter* over the control in 1st and 2nd seasons, respectively. The favorable effect of biofertilizers on total yield and its components could be explained through the great role of these fertilizers in enhancing plant growth rate, which exert direct effect on the yield and its components. Similar results were obtained by Yaso *et al*. (2007) on onion, Nour *et al.* (2010), Abdel-Razzak and El-Sharkawy (2013) on garlic. As for effect of the interaction, it is obvious from the data in Table 6 that the interaction treatments between mineral nitrogen levels and N_2 fixing bacteria reflected significant effect on all yield components, these results were matched during both seasons of study. Generally, it is noticed that the interaction between 100% mineral nitrogen and inoculation with the combination of the two fixing bacteria was the superior interaction followed by 75% mineral nitrogen plus inoculation with the combination of the two fixing bacteria. On the other side, the interaction between 50% mineral nitrogen and untreated plants with biofertilizers recorded the lowest value of yield and its components. These results are in agreement with those of Nour *et al.* (2010) on garlic.

Storage experiment

Weight loss percentage:

It is obvious from the data in Table 7 that application of 100% mineral N/fed. significantly increased weight loss (%) of green onion plants as compared to other levels of mineral nitrogen, the weight rate reached to 9.89 and 9.13 in the 1st and 2nd season, respectively. The increases in weight loss (%) of green onion plants due to application of high rate of nitrogen may be

owed to the high content of moisture in plants. Similar results were found by El-Tantawy and El-Beik (2009) on onion.

Concerning the inoculation of N_2 fixing bacteria, the results show that treating onion seeds with N_2 fixing bacteria significantly reduced weight loss (%) in the stored plants as compared to untreated plants, these results are true in the 1st season only. On the contrary, untreated plants significantly reduced weight loss (%) in the 2nd season. Such results may suggest that beneficial effects of biofertilizers on weight loss may referring to one or more of the following mechanisms; N-fixation facilitate, promoting substances or organic acids for production of plant growth, enhancing nutrient uptake for storage organs. Similar results were obtained by (Abdel-Razzak and El-Sharkawy 2013) on garlic who found that inoculation with biofertilizers decreased weight loss%. As for cold storage period, it is clear that there was a considerable increase in weight loss (%) of stored green onion plants when the cold storage period was prolonged, whereas the maximum loss was occurred at the end of cold storage period (20days) 13.24 and 12.22 % in 1st and 2nd season, respectively. This continuous loss in weight during cold storage resulted from the loss of water by transpiration and dry matter by respiration (Atta-Aly, 1998) on green onion. Similar results were obtained by Emam (2009) on green onion and Ismail and Mohamed (2014) on sweet pepper.

Regarding the effect of interaction between mineral nitrogen levels and N_2 fixing bacteria, results in Table 7 show that the highest value of weight loss (%) was recorded from the plants which were fertilized with 100% mineral N and untreated with N_2 fixing bacteria, while fertilization of green onion plants with 50% mineral N and inoculation with *Azotobacter* gave the lowest value. These results are true in 1st season only, whereas, in the 2nd season fertilization the green onion plants with 100 % mineral N and inoculation with *Azospirillum* plus *Azotobacter* significantly increased weight loss (%) while, the lowest value was recorded from untreated plants with N_2 fixing bacteria and fertilized with 100% mineral N (90kg/fed.).

The interaction between mineral N levels and cold storage period showed significant effect in both seasons, the lowest value of weight loss (%) at the beginning of cold storage period (5days) were noted by fertilization with 50% mineral nitrogen, while the highest value (14.57 and 12.73%) in the 1st and 2nd seasons, respectively, was occurred at the end of cold storage periods (20 days) by fertilizing plants with 100% mineral nitrogen in both seasons of study. With respect to the interaction between cold storage periods and N_2 fixing bacteria, the same results in Table 7 show that inoculation of green onion plants with *Azotobacter* recorded the lowest value of weight loss (%) at the beginning of cold storage period, while untreated plants gave the highest value of weight loss (%) at the end of cold storage periods (20 days) in the first season only. Whereas, in the second season, untreated plants recorded the lowest value of weight loss (%) at the beginning of cold storage period, while the highest value was recorded at the end of cold storage periods (20days) from green onion plants which were inoculated by the combination between *Azospirillum* plus *Azotobacter*.

Regarding effect of the interaction among mineral nitrogen levels, N_2 fixing bacteria and cold storage periods, it is clear that the lowest value of weight loss (%) at the end of cold storage periods (20 days) were noted by fertilizing green onion plants with 75% nitrogen and inoculated with *Azotobacter* that gave 11.43 and 11.06% in the 1^{st} and 2^{nd} seasons, respectively.

Total Chlorophyll:

It is obvious from the data in Table 8 that fertilization of green onion plants with 100% mineral nitrogen significantly increased total chlorophyll followed by 75% without significant differences between them in the first season, while mineral nitrogen levels did not reflect significant effect on total chlorophyll in the second season. The increment in total chlorophyll due to application of high rate of nitrogen may be attribute to the pronounced role of nitrogen in plant metabolism and it is a constituent of phototosynthesis (Reddy and Reddi, 2002). Similar results were found by Nour *et al.* (2010) on garlic.

As for inoculation with N_2 fixing bacteria, the results show that pre-sowing inoculation of onion seeds with N_2 fixing bacteria significantly increased total chlorophyll in the stored plants as compared to untreated plants. The highest value of total chlorophyll was recorded by the combination between *Azospirillum* plus *Azotobacter* followed by *Azotobacter* without significant differences between them. The promoting effect of biofertilizer treatments on chlorophyll pigments content may be related to the role of the same symbiotic and non symbiotic N_2-fixing in producing of phytohormones or improving the availability and acquisition of nutrients or both which promoted the vegetative growth. These results are in harmony with those obtained by and Nour *et al.* (2010) on garlic.

With respect to cold storage period effect, it is clear from the same data in Table 8 that total chlorophyll was significantly decreased as the cold storage periods prolonged, where the maximum total chlorophyll was occurred at the harvesting time, the minimum value was occurred at the end of cold storage periods (20days). The reduction in chlorophyll content with the elapse of cold storage period may be due to the destruction of the chlorophyll and transformation of chloroplasts to chromoplasts by chlorophyllase activity (Hulme, 1970). These results are in agreement with

those obtained by Emam (2009) on green onion and Mohamed (2014) on pea.

With respect to effect of the interaction between mineral nitrogen levels and N_2 fixing bacteria, the results show that fertilization with 100% mineral nitrogen plus inoculation with the mixture of the two N_2 fixing bacteria recorded the highest value of total chlorophyll (62.5 and 58.9 SPAD) followed by 75% which gave 60.8 and 58.8 SPAD without significant differences between them.

Concerning the effect of interaction between mineral nitrogen levels and cold storage periods, it is clear from the same data in Table 8 that there was a considerable decrease in total chlorophyll content of stored green onion plants as the storage period prolonged, whereupon, the minimum total chlorophyll was occurred at the end of cold storage periods (20 days) by fertilizing onion plants with 50% mineral nitrogen.

The interaction between N_2 fixing bacteria, and cold storage periods show that pre-sowing inoculation of onion seeds with the combination between *Azospirillum* plus *Azotobacter* recorded the highest value of total chlorophyll 68.9 and 70.6 SPAD unit at the harvesting time in the 1st and 2nd seasons, respectively. These results are true in both seasons of study. Regarding the effect of interaction among mineral nitrogen levels, N_2 fixing bacteria and cold storage periods, generally it is clear that the highest value of total chlorophyll at the end of cold storage periods (20days) were noted by fertilizing green onion plants with 100% mineral nitrogen which inoculated with the mixture of *Azospirillum* plus *Azotobacter* (56.2 and 47.9 SPAD) followed by 75% N and inoculation with *Azospirillum* plus *Azotobacter* (55.3 and 49.3 SPAD) in the 1st and 2nd seasons, respectively.

Total soluble solids (TSS):

Concerning the effect of mineral nitrogen levels, it is obvious from the data in Table 9 that fertilizing green onion plants with the different tested levels of mineral nitrogen did not reflect any significant effect on total soluble solids in both seasons of study. The same results show that pre-sowing seed inoculation with the mixture of *Azospirillum* plus *Azotobacter* significantly increased TSS (%) which recorded 11.2 and 11.1 % in the 1st and 2nd seasons, respectively. On the other hand, the lowest value of TSS (%) was recorded from untreated plants in both seasons of study. As for cold storage periods effect, it is clear from the results in Table 9 that, TSS (%) of green onion plants were decreased as the duration of cold storage increased, the results demonstrated that TSS (%) of green onion plants were significantly increased at the beginning of cold storage and then decreased with the prolongation of the storage period in the two seasons. The increase in TSS (%) at harvesting time might owe much to the higher rate of

moisture loss through transpiration, however the reduction in TSS (%) during the end of cold storage periods might owe much to the higher rate of sugar loss through respiration than water loss through transpiration (Wills *et al.*, 1998). Similar results were obtained by Emam (2009) on green onion and Ismail and Mohamed (2014) on sweet pepper.

The interaction between mineral nitrogen levels and N_2 fixing bacteria show that fertilization of green onion plants with 75% mineral nitrogen and inoculation with the mixture of *Azospirillum* plus *Azotobacter* recorded the highest value of total soluble solids (11.89 and 11.74%) in the 1st and 2nd seasons, respectively. On the contrary, the lowest value of TSS% was recorded from the plants which fertilized with 50% N and uninoculated with N_2 fixing bacteria in both seasons.

With regard to effect of the interaction between mineral nitrogen levels and cold storage periods, the same results in Table 9 show significant effect on TSS% in both seasons, the lowest value was occurred at the end of cold storage periods (20days) and fertilizing green onion plants with 50% mineral nitrogen. While, the highest value was recorded at harvesting time and application of 100% mineral nitrogen followed by 75% without significant differences between them in both seasons.

Respecting to the interaction between N_2 fixing bacteria and cold storage periods, results showed significant effect on TSS(%) in both seasons, the highest value of TSS(%) was recorded by inoculation of green onion plants with the combination between *Azospirillum* plus *Azotobacter* at harvesting time, followed by inoculation with *Azotobacter* at harvesting time without significant differences between them. Regarding effect of the interaction among the three factors, it is clear also from the data in Table 9 that the highest values of total soluble solids at the end of cold storage periods (20days) were noted by fertilizing green onion plants with 75% mineral nitrogen and inoculation with the combination between *Azospirillum* plus *Azotobacter* which gave 11.1 and 10.8 % in the 1st and 2nd seasons, respectively.

Leaf extension:

Regarding effect of mineral nitrogen levels, the results listed in Table 10 clearly show that fertilization with 100% mineral nitrogen increased significantly leaf extension of the cut end (1.90 and 1.92cm) in the 1st and 2nd seasons, respectively, followed by application of 75% without significant differences between them. On the other side, the lowest value of leaf extension was noted by the lowest mineral nitrogen level (50%) in both seasons of study.

As for inoculation with N_2 fixing bacteria, the same results in Table 10 show that inoculation of green onion plants with N_2 fixing bacteria did not

reflect significant effect on leaf extension of the cut end, these results are true in both seasons of study.

With respect to cold storage periods, it is clear from the results in Table 10 that leaf extension of the cut end reached to 0.38 cm when green onion plants were stored for five days. Moreover, a continuous increase in leaf extension was evident when storage period was extended to 20days and leaf growth reached to 3.27 and 3.07 cm in the 1st and 2nd seasons, respectively. Such an increase in leaf extension negatively effected the market quality. Similar results were found by Emam (2009) on green onion.

Concerning the effect of interaction between mineral N levels and N_2 fixing bacteria, the results show that fertilizing green onion plants with 100% mineral nitrogen plus inoculation with N_2 fixing bacteria significantly increased leaf extension of the cut end without significant differences among them. Generally the highest value was recorded by uninoculated plants, followed by inoculation with *Azospirillum*, while the lowest value was recorded by fertilization with 50% N and untreated with biofertilizers followed by inoculation with *Azospirillum* without significant differences between them in both seasons of study. Regarding the effect of interaction between mineral nitrogen levels and cold storage periods, it is clear from the same data that there was a considerable increase in leaf extension of the cut end of stored green onion plants as the storage period prolonged whereupon, the minimum value was occurred at the beginning of cold storage periods (5days) by fertilizing onion plants with 50%, while, the maximum value was obtained at the end of cold storage periods (20days) by fertilizing onion plants with 100N.

The interaction between N_2 fixing bacteria and cold storage periods show that inoculation of green onion seeds with *Azospirillum* recorded the lowest value of leaf extension of the cut end at the beginning of cold storage periods (0.37 and 0.38cm) in the 1st and 2nd seasons, respectively, while the highest value was obtained at the end of cold storage period (20 days) by treating with *Azospirillum* plus *Azotobacter*. Regarding the effect of interaction among the three factors, it is clear that the highest value of leaf extension at the end of cold storage periods (20days) was, generally, noted by fertilizing with 100N plus inoculation with *Azospirillum*, while the lowest value was occurred at the beginning of cold storage periods (5 days) after application of 50% N and untreated with biofertilizers.

Curvature score:

Regarding the effect of mineral nitrogen levels, it is obvious from the data in Table 11 that fertilization of green onion plants with the different rates of mineral nitrogen did not reflect significant effect on curvature score in both seasons. With respect to N_2 fixing bacteria the same results show

that inoculation green onion seeds with N_2 fixing bacteria significantly affected curvature score in second season only. Untreated seeds gave the highest value while, inoculation with *Azospirillum* recorded the lowest value of curvature score. As to cold storage periods, it is clear from the results in Table 11 that continuous increases in curvature score were evident where storage period was extended to 20 days and curvature score reached to 2.44 and 3.50 in 1st and 2nd seasons respectively, such an increase in curvature score negatively affected the market quality. Similar results were found by Emam (2009) on green onion.

Concerning the effect of interaction between mineral N and N_2 fixing bacteria, the same results show that the interaction between mineral N levels and N_2 fixing bacteria had significant effect on curvature score. The highest value was recorded by the interaction between 100% N and untreated seeds with biofertilizers, while the lowest value was recorded by the interaction between 50% N and inoculation with *Azospirillum* in the two seasons. Regarding the effect of interaction between mineral N levels and cold storage periods, it is clear from the same data in Table 11 that, there was a considerable increase in curvature score of stored green onion plants as the storage period prolonged, whereas, the minimum value was occurred at harvesting time by fertilizing onion plants with 50% N while, the maximum value was obtained by fertilization with 100%N at the end of cold storage periods (20days) in both seasons.

The interaction between N_2 fixing bacteria, and cold storage periods show that the highest value of curvature score was recorded at the end of cold storage periods from untreated seeds with biofertilizers which gave 2.56 and 3.89 in the first and second seasons, respectively. With respect to the interaction among the three factors it is clear from the data in Table 11 that the highest value of curvature score at the end of cold storage periods (20 days) was noted by fertilization green onion plants with 100% mineral N and untreated with biofertilizers in both seasons of study.

Conclusively, from the previous results of this investigation, it could be concluded that substituting the inorganic 100% N fertilizer with 75% mineral N plus inoculation with *Azospirillum* plus *Azotobacter* was sufficient to produce the highest vegetative growth parameters, root system and yield and its components of onion during cold storage at 0 ^{o}C and 90-95 RH for 15 days. This substitution of the inorganic N may help in lowering environmental pollution and overcoming the problems of high prices of chemical fertilizers by decreasing the total cost of production.

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تأثير مستويات من التسميد النيتروجينى المعدنى والبكتريا المثبتة للنيتروجين على النمو والمحصول والقدرة التخزينية لنباتات البصل الأخضر أثناء فترات التخزين المبرد

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أجريت تجربتان حقليتان خلال شتاء موسمى ٢٠١٢/٢٠١٣ ، ٢٠١٣ / ٢٠١٤ فى مزرعة التجارب البحثية - بمحطة بحوث البساتين بالقصاصين - مركز البحوث الزراعية ، محافظة الأسماعيلية ، ومعمل قسم بحوث تداول الخضر بمعهد بحوث البساتين - مركز البحوث الزراعية. لدراسة تأثير إستبدال وإحلال التسميد الحيوى الذى يحتوى على سلالتين من مثبتات النيتروجين (أزوتوباكتر كروكوكم ، أزوسبيريلم ليبوفيرم) بديلا عن التسميد النيتروجينى المعدنى على النمو ، والوزن الجاف ، والمجموع الجذرى ، والمحصول ومكوناته للبصل الأخضر صنف جيزة ٢٠ النامى تحت ظروف الأراضى الرملية بإستخدام نظام الرى بالتنقيط ، كما تهدف الدراسة أيضا إلى دراسة تأثير المعاملات السابقة الذكر على نباتات البصل الأخضر

أثناء فترات التخزين المبرد (صفر ، ٥ ، ١٠ ، ١٥، ٢٠ يوما) من بداية فترة التخزين على درجة صفر° مئوية ورطوبة جوية نسبية من ٩٠- ٩٥%.

سجلت معاملة تسميد البصل الأخضر بالنيتروجين المعدنى بمعدل ١٠٠% من المعدل الموصى به أعلى القيم بالنسبة للصفات المورفولوجية ، و قياسات المجموع الجذرى ، والمحصول ومكوناته ، والنسبة المئوية للفقد فى الوزن ، والكلوروفيل الكلى ، وإستطالة الأوراق وكذلك معامل الإنحناء، يليها معاملة التسميد بمعدل ٧٥%.

كما أعطت معاملة التلقيح بالأزوتوباكتر + أزوسبيريلم أعلى القيم بالنسبة للنمو الخضرى ، والمحصول ومكوناته وكذلك صفات الجودة أثناء فترات التخزين المبرد.

سجلت معاملة التفاعل بين التسميد المعدنى بمعدل ١٠٠% أو ٧٥% والتلقيح بالأزوتوباكتر + أزوسبيريلم أفضل القيم بالنسة لقياسات النمو ، والمحصول ومكوناته بدون فرق معنوي بينهما. كما أوضحت النتائج أن التخزين المبرد لنباتات البصل الأخضر على درجة صفر° مئوية ورطوبة جوية نسبية من ٩٠- ٩٥% لمدة ٢٠ يوما أدى إلى حدوث زيادة فى كل من النسبة المئوية للفقد فى الوزن ، وإستطالة الأوراق ، ومعامل الإنحناء وذلك بزيادة فترة التخزين ، بينما سجل إنخفاض معنوي فى كل من الكلوروفيل الكلى والنسبة المئوية للمواد الصلبة الذائبة الكلية بزيادة فترة التخزين، و سجلت معاملة التفاعل بين التسميد المعدنى بمعدل ١٠٠% أو ٧٥% والتلقيح بالأزوتوباكتر + أزوسبيريلم والتخزين المبرد لنباتات البصل الأخضر لمدة ١٥ يوما على درجة صفر° مئوية ورطوبة جوية نسبية من ٩٠- ٩٥% أفضل القيم للنسبة المئوية للفقد فى الوزن ، والكلوروفيل الكلى، والنسبة المئوية للمواد الصلبة الذائبة الكلية ،وإستطالة الأوراق ، وكذلك معامل الإنحناء.

التوصية: نستنتج من النتائج السابقة بأنه يمكن إستبدال التسميد النيتروجينى المعدنى بمعدل ٧٥% مع التلقيح بالأزوتوباكتر والأزوسبيرليم بديلا عن التسميد النيتروجينى المعدنى بمعدل ١٠٠% كان كافيا للحصول على أعلى معدل للنمو الخضرى ونمو الجذور ، والمحصول ومكوناته كماسجل أفضل القياسات أثناء فترة التخزين المبرد لبناتات البصل الأخضر على درجة حرارة صفر° مئوية ورطوبة جوية نسبية من ٩٠- ٩٥% لمدة ١٥ يوما.

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