*Egypt. J. Microbiol.* **Vol. 56**, pp. 35-51 (2021)

EgyptianJournalofMicrobiology
http://ejm.journals.ekb.eg/

# Effect of Bio-fertilization and Nano-silica on Maize Plant at New Valley

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A FIELD experiment was carried out on maize at the New Valley to examine its response to some bio-fertilizers applied separately or with spray with Nano-silica K. The three bio-fertilizers are *Azospirillum brasilense*, *Azospirillum lipoferum,* and mycorrhiza, in addition to their interactions and a control treatment (without biofertilizer). All 16-combination treatments were arranged in split plot technique in three randomized complete blocks. The objective of this work was to examine the mutual influences of all applied treatments on some maize growth parameters. The treatment comprised of inoculation with ½ *Azospirillum brasilense* + ½ *Azospirillum lipoferum* + mycorrhiza + spraying of Nano silica—K proliferated the highest significant total bacterial counts, total *Azospirillum brasilense*, total *Azospirillum lipoferum*, total mycorrhizal counts, dehydrogenase activity, phosphatase enzyme, and root colonization by mycorrhiza in both years of study. *Azospirillum lipoferum* significantly showed better performance in all measured properties than *Azospirillum brasilense*. Spray of the aerial parts of maize plant with Nano-silica K could indirectly affect counts of biological inoculants in the soil under maize plants at the New Valley in both years of study. It can be recommended to add Nano-silica K to the rhizosphere to practice its direct influence on the activities of soil micro fauna.

**Keywords:** Arbuscular mycorrhiza, *Azospirillum brasilense*, *Azospirillum lipoferum,* Maize, Nano silica K, The New Valley.

## Introduction

Fulchieri et al. (1993) found that maize seedlings were inoculated with three strains of *Azospirillum lipoferum* or cultured in different concentrations of indol-3-acetic acid (IAA) or gibberellin $A_3$ ($GA_3$). After 48hrs., root length, root surface area, root dry weight, and shoot dry weight were measured in all treatments. Gibberellin content was evaluated in selected roots of all seedlings. They found that the three strains of *A. lipoferum* significantly enhanced root hair growth and density than IAA and $GA_3$ soaking treatments. Their finding points to that *Azospirillum lipoferum* significantly contributed to the establishment of a vigorous root growth proliferating for healthy plant growth, thence a greater potential for enhanced grain yield.

Shekh (2006) fertilized maize with various sources of bio-fertilizers including nitrogen fixers, phytostimulators, phosphate solubilizing bacteria, and plant growth promoting rhizobacteria. He reported that all sources were beneficial to attain good plants growth and grain yield. In addition, Hungria et al. (2010) stated that using an inoculant containing bacteria that promote plant growth is likely to increase in the coming years due to higher costs of fertilizers, concerns over pollution and emphasis on sustainable agriculture. They recommended *Azospirillum* strains for maize (*Zea mays* L.) fertilization. They evaluated nine *Azospirillum* strains being applied to seeds as peat-based inoculants. Some *A. brasilense* strains increased grain yields of maize by 24–30% in relation to non-inoculated controls. And that, two *A. lipoferum* strains were tested in two separate

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Received 31/1/2021; Accepted 14/3/2021
DOI: 10.21608/ejm.2021.60725.1187

experiments and promising results were obtained. They attributed their findings to general increases in the uptake of several macro and micronutrients and not specifically to biological nitrogen fixation. All experiments on maize received only a low starter dose of N-fertilizer at sowing of 24kg of N $ha^{-1}$. This result points to a large saving in the amount of N-fertilizers; i.e. big drop in fertilizer cost and lowering the environmental pollution. They also stated that the obtained yields were compatible with Brazilian mean yields. And that, they could identify the first *Azospirillum* strains that are authorized for the production of commercial inoculants in Brazil.

Ferreira et al. (2013) testified the combination of nitrogen and *Azospirillum brasilense* in sandy soils of the Brazilian Cerrado on maize yield and shoot dry weight (SDW). They reported that inoculation with *A. brasilense* gave comparable yield to the nitrogen treatment. And that, the grain production was increased by 29% in the treatment of *A. brasilense* together with mineral nitrogen fertilization as compared to nitrogen fertilization alone.

In the same respect, Morais et al. (2016) applied diazotrophic microorganism of *Azospirillum brasilense* to the seed in the furrow when planting maize at the doses of 0, 100, 200, 300 and 400mL $ha^{-1}$ in combination with nitrogen fertilizer at the rates of 40, 100, 200 and 300kg $ha^{-1}$. They found that the dose of 200mL $ha^{-1}$ *Azospirillum* was noteworthy for grain production at the Cerrado region of Brazil irrespective of nitrogen fertilizer application level. They recommended that the lower the level of N fertilizer application, the greater he benefit obtained from the bio-fertilizer; let's say 40-100kg $ha^{-1}$.

Rossel & Tarradellas (1991) stated that the inhibition of dehydrogenase activity (DHA) correlated with depression of ATP content ($r$= 0.82). Air-drying, remoistening, and substrate addition had little influence on the depression of DHA and of the ATP content. In other words, irrigation and drying of plants does not affect the depression of DHA and of the ATP content. In addition, short-term DHA, as a substrate-induced maximum initial activity, appears mainly to reflect the biomass of soil microflora. The measurement of DHA appears to be a suitable low-cost and sensitive tool for assessing the biomass size in response to the surrounding effects in the environmental conditions, such as pollutants and other chemicals.

With regard to mycorrhizal influence on plant growth, Zhang et al. (2013) showed that the mean value of soil $CO_2$ fluxes from 08:00 to 10:00am could represent its daily mean value in summer period (June–August) and that from 09:00am to 12:00pm for the rest seasons of a year. Annual cumulative soil $CO_2$ fluxes were significantly higher than those in the treatments with non-manure addition. However, the treatments with manure applications represented a carbon sink in the soil (carbon output/input ratio < 1.0), which demonstrated potential for carbon sequestration. They also emphasized the role played by arbuscular mycorrhiza in controlling the fluxes of $CO_2$ between the aerial and underground parts of maize.

Radwan & Nassar (2011) found that VA-mycorrhizal combined with bio-fertilizer cerealen and 105 kg N/ha was the best treatment to obtain the highest growth attributes, yield, and yield components. Mohammed (2012) showed that maize plants exhibited the highest significant plant height, LAI, 500 kernel weight, biological yield, yield, harvest index (HI), and root-shoot ratio in response to phosphorus bio-fertilizer in the presence of different doses of nitrogen and $P_2O_5$.

As long as mycorrhiza is a symbiotic association between a fungus and the roots of a vascular plant, Muthukumar (2017) studied the influence of arbuscular mycorrhizal (AM) fungus *Scutellospora calospora* on root architecture, growth, nutrient uptake, root phosphatase activity and mycorrhizal dependency of maize in 0-5% rock phosphate (RP) amended phosphorus (P) deficient soil. RP amendment significantly increased total root length, number of roots in different orders, and root hair diameter of AM plants. The AM fungus positively enhanced maize growth and nutrient uptake. Acid and alkaline phosphatase activities were higher for AM plants in RP amended soils. They stated that AM fungus inoculation along with RP amendment could substitute chemical fertilizers and rendered the P in RP available to the plants.

Salgado et al. (2017) measured shoots dry matter (SDM), roots dry matter (RDM), mycorrhizal colonization and accumulation of calcium, zinc

and phosphorus in the SDM at maize flowering in response to inoculation with five different arbuscular mycorrhizal fungi at Cerrado, Brazil. They concluded that inoculation with mycorrhizal fungi demonstrated different effects for the various parameters evaluated in maize.

Shamsul et al. (2012) suggested a positive correlation between proline accumulation and plant stress, where proline plays a highly beneficial role in plants exposed to various stress conditions. They also stated that proline acts as a metal chelator, an antioxidative defense molecule and a signaling molecule. Therefore, a stressful environment results in an overproduction of proline in plants.

Yuvakkumar et al. (2011) studied the effect of Nano-silica on maize crop improvement. They mixed Nano-silica powders with soil at different concentrations along with control and conventional silica under in vitro and *in vivo* conditions. In *in vitro*, the Nano-silica increases seed germination (2–11%), water usage efficiency (up to 53%), and total chlorophyll content (13–17%) of maize crop. In in vivo, influence of Nano-silica was analyzed on basic parameters such as stem height, stem width, number of leaves, and silica content. The effect of Nano-silica on maize crop was found to be enhanced in all aspects. Also, Amer & El- Emary (2018) reported that Nano-fertilizers (different concentrations with Nano-silica) can significantly improve soil productivity, enhance nutrition use efficiency, and protect plants from environmental stress.

With respect to Nano-K silica spray on maize plants, Guntzer et al. (2012) reported that silicon (Si) has been found in significant concentrations in plants, but it is generally not considered as an essential element. Silicon can mitigate environmental stresses and soil nutrient depletion and as a consequence is an alternative to the extensive use of phytosanitary and NPK fertilizers for maintaining sustainable agriculture. In other words, N, P, and K can be impregnated on Nano silica and sprayed on to the aerial parts of the plants.

Laane (2018) stated that foliar sprays with silicates increase growth and yield and decrease biotic and abiotic stresses. He also added that despite being limited the available data on foliar silica-Nano sprays show a tendency to decrease biotic stress and to stimulate a limited increase in growth and yield.

With respect to phosphatase enzyme activity, Machado & Furlani (2004) reported that it is a physiological characteristic related to plant efficiency in relation to P acquisition and utilization. They pointed to finding positive and/or negative correlations between phosphatase activity and P-efficiency characteristics, specific for the genotypes, not allowing inference on a general and clear association between root-secreted phosphatase and dry matter production or P acquisition under low P conditions.

## Materials and Methods

*Agricultural experiment*

A field experiment was conducted in newly reclaimed sandy soils at El-Monira village, El-Kharga Oasis of the GIS indices of 30.53 longitude, 25.45 latitude and 78.8m altitude at the New Valley Governorate during two successive cropping seasons of 2016 and 2017. The major objective of the current research was to study the response of maize plants to bio fertilizers which included inoculation with *Azospirillum brasilense, Azospirillum lipoferum,* mycorrhiza, and their mixtures, in addition to a control without bio inoculation, as well. All bio-fertilizer treatments were applied either separately or with Nano silica spraying.

*Microorganisms*

Bacterial isolates recruited in the current experiment were isolated from the experimental soil at newly reclaimed sandy areas that was previously mentioned. The isolated bacteria were identified as being *Azospirillum brasilense, Azospirillum lipoferum* according to Krieg & Holt (1984). Bacterial concentration of the applied suspensions was adjusted to $10^8$ CFU/ml for the two microbial treatments of *Azospirillum brasilense, Azospirillum lipoferum* prior to application to the treated grains. With respect to mycorrhiza, spores were collected from the zero-to-30cm soil layer. The obtained soil samples were exposed to wet sieving and decanting technique according to the method descried by Gerdemann & Nicolson (1963) and Faramawy (2013). This technique preludes to suspend 100g of soil into a 2L container and add 1.5L of water. Vigorously mix the suspension to free the spores from the soil and roots. For fungal colonization in roots, clean root sample was blended for 1min in 300mL of water to free the spores from roots. Next, the supernatant solution containing the spores was decanted through standard sieves. A

425µm pore size over a 45µm pore size sieves was used. The content of the top sieve is examined for sporocarps that may be up to 1 mm in diameter. For clay soils, it is advisable to repeat the decanting and sieving procedure with the settled soil. Roots were collected from the larger mesh sieve for evaluation of internal colonization. The sieving's retained on the other different sieves were washed into separate petri dishes for further observations or purification by sucrose centrifugation. Thus, it could be possible to obtain a mycorrhizal suspension that can be recruited as a source for inoculation of maize grains with mycorrhiza. After inoculation of grains with mycorrhiza and cultivation, estimation of mycorrhizal colonization can be achieved by using the method described by Trouvelot et al. (1986). Number of mycorrhizal spores induced per treatment was to be reported as No. of spores/100g soil.

Regarding the Nano-silica used in the current research work, it was provided by National Research Center (NRC) at Giza governorate. It was characterized by specific surface area of 300-330$m^2$ $g^{-1}$, pH 4.0-4.5, and mean diameter 10nm.$g^{-1}$

Maize grains were to be wetted before planting with bacterial suspensions at the rate of 250mL suspension/5kg grains for three hours before planting. Carboxy methylcellulose 0.5% was used as an adhesive agent; i.e. spreading agent. The previous pre-planting treatment with microbial suspensions represents half dose of the bio-fertilizer treatments. The other half was applied in the form of spray using a back-mounted sprayer to soil down the plants aging one month after seeding.

Regarding to phosphorus fertilizer application, it was applied at the rate of 45kg P/fed with the applied organic manure which was added at the rate 20$m^3$/fed. 18 days prior to seeding. The applied amounts were metered per dripper line and were to be applied in the furrow that was manually dug, then the furrow was filled with the dugout soil. This practice was achieved the same for all treatments.

All plants received solid potassium sulfate (50% $K_2O$) after 45 days from applied 5-7cm away from the plants' stems to avoid plant burn damage. Half of the plants received the remaining recommended amount of K as potassium sulfate in the previously mentioned way via the soil after 60 days from sowing. At the same time (60 days from sowing) the other half of the plants received potassium silicate as spray onto the aerial parts of the plants.

Farmyard manure was broadcast applied and incorporated into the soil surface layer by hand-hoeing, and then the dripper lines were straight back to their places. A quick false irrigation was applied on to the experimental dripper lines through the drip irrigation network 18 days before seeding to allow the exposure of weeds that may be existing in the soil and the added farm manure to combat them.

Seeding rate was 5kg maize grains/feddan (1feddan= 0.42ha). On the seeding day, 3-4 grains were to be placed in hills 30cm apart along the dripper lines. After germination, number of plants was thinned to 2 plants per hill to achieve a full 100% stand at the beginning of the cultivation season. Owing to the fine textured soil nature (around 48% as silt + clay), drip irrigation was to be practiced every six-day period with short application period each time to avoid loss of soluble fertilizers with the soil water percolating down the rhizosphere, i.e. beyond the plants' root system.

*Some physical and chemical analyses of both the adopted soil and irrigated water*

Some physical and chemical analyses of the experimental field soil were achieved according to Page et al. (1982) and presented in Table 1. This table points to a vigorous salt stress (EC= 8.21dS.$m^{-1}$) and alkaline reaction (pH = 8.64) with the domination of Na and Cl ions. There is an alkalinity threat to plants grown in such soil. They need enough organic fertilizers to combat such alkalinity hazard and correct for good plant nutrition. Fortunately, the irrigation water does not threaten the grown plants owing to their low pH, EC, high Ca and Mg, low Na and Cl. In addition, chemical analyses of irrigation water were depicted in the same table. The latter analyses exhibited that the irrigation water had a pH 7.5 and an EC 1.46 dS.$m^{-1}$.

*Test plant*

Grains of the adopted test plant maize hybrid cultivar, namely, three-way cross 310 (T.W. C. 310) (*Zea mayz* L.) were purchased from the Crops Research Institute, Agricultural Research Center, Giza, Egypt.

**TABLE 1. Analysis of experimental soil to a 30cm depth and irrigation water**

| Physical analysis % | | | Soil texture |
|---|---|---|---|
| Sand | Silt | Clay | |
| 52.95 | 21.51 | 25.54 | Sandy clay loam |

| Chemical analysis | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | E.C dS.m$^{-1}$ | $CaCO_3$ % | O.M % | T.N % | Cations meq/L | | | | Anions meq/L | | | |
| | | | | | $Ca^{+2}$ | $Mg^{+2}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{-2}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{-2}$ |
| 8.64 | 8.21 | 4.32 | 0.59 | 0.09 | 25.2 | 7.9 | 43.4 | 5.3 | 0.9 | 15.9 | 50.9 | 12.2 |

| Chemical analysis of irrigation water | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| pH | E.C dS.m$^{-1}$ | $Ca^{+2}$ | $Mg^{+2}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{-2}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{-2}$ |
| 7.5 | 1.46 | 5.42 | 3.24 | 5.43 | 0.27 | 1.65 | 2.18 | 4.2 | 6.16 |

*Plant growth promoting properties of bacterial isolates*

*Agronomic data recoded*

*Soil biological activity:* Enumeration of microorganisms in soil samples was carried out by the most probable number (MPN) technique. One milliliter successive dilutions of $10^5$ and $10^4$ was attained. Soil samples were to be transferred to test tubes containing semi-solid NFB medium for enumeration *Azospirillum brasilense, Azospirillum lipoferum* isolates, respectively. Tubes and plates were then incubated under suitable temperature. Microorganisms were identified based on cultural, morphological and biochemical characteristics as per Bergey's Manual of Systematic Bacteriology.

Bacterial population= (MPN value × middle dilution × middle dilution used)/ (Dry weight of the soil sample)

Mycorrhizal colonization and plant biomass analysis. All roots were thoroughly rinsed with tap water before drying, and weighed subsamples of fresh roots were used for mycorrhizal colonization assessment by the gridline intersect method (Giovannetti & Mosse, 1980) after clearing with 10% (m/m) KOH and staining with acid fuchsine (Phillips & Hayman, 1970).

*Determination of microbial activity:* Counts of microorganisms were estimated by the dilution plate technique methods (Becky et al., 2001). The following microbial analyses; total microbial count (Counts x $10^5$ CFU g$^{-1}$ dry soil) and *Azospirilla* densities; *lipoferum* and *brasilense* (Counts x $10^4$ CFU g$^{-1}$ dry soil) were carried out in all soil samples according to Pious et al. (2015). Total Mycorrhiza (Counts x$10^4$ number of spores Kg$^{-1}$ soil) of Arbuscular mycorrhizal fungi (AMF) spores in the soil was determined by wet sieving (Gerdemann & Nicolson, 1963)**.**

Dehydrogenase activity (µg TPF g$^{-1}$ dry soil 24h.) in the rhizosphere soil was determined according to Pramer & Schmidt (1964) and Thalmann (1967). Production of ammonia was determined according to Cappuccino & Sherman (1992).

Phosphatase activity has specified in the soil by using the method according to Tabatabai & Bremner (1969).

*Soil analyses*

Soil samples were collected from each dripper line at the same time of plant sampling (at what exactly time), air-dried, passed through a 2mm sieve and kept for physical and chemical analyses. Particle size distribution was determined using the pipette method according to Jackson (1973). Electrical conductivity (EC) and soil pH was determined in a 1: 2.5 soil to water extract using conductivity meter and Beckman pH meter, respectively according to Jackson (1973). Organic carbon content was determined by Walkely and Black's wet oxidation method (Walkley & Black, 1984). Available potassium was extracted by neutral normal ammonium acetate method and measured by flame photometer. Available P was extracted using 0.5 M $NaHCO_3$ at pH 8.5 according to Olsen et al. (1982) and measured calorimetrically using the chlorostannus phosphomolybdic-sulfuric acid method as described by Jackson (1973).

*Chemical composition of plants and grains*

Grains nitrogen content was determined by

the modified microkjeldahl method as described by Peach & Tracey (1956). Phosphorus percentage was estimated by ascorbic acid according to the method reported by Bender & Wood (2000). Potassium was determined by using the flame photometer method as described by Knudsen et al. (1982).

*Yield and yield components*

At harvest, plant height (cm), grain yield, and harvest index (1fed.=0.42ha). In addition, oil production (g/plant) was also assessed and calculated as kg/fed. To evaluate effects of the applied treatments on seed quality, seed mineral content of N, P, and K were achieved.

*Statistical analysis*

All dripper lines were divided into three groups of drippers according to the expected drop in the driving head along the end tail of lines. The three groups were to be sampled for three replicates, respectively. The obtained data were exposed to the analysis of variance (ANoVA) according to the statistical design of split plot technique in randomized complete blocks using SPSS (2014) software package. The Duncan least significant range (LSR) will be recruited for comparing the mean values of variables and their interactions using alphabetical letters. In other words, any two mean values sharing one letter are to be considered not significantly different at 5% level.

## Results and Discussion

In the following, the obtained date will be exhibited and discussed under: 1. Effects of bio-fertilizers on all studied traits will be presented and discussed first, 2. Effects of Nano silica potassium, and 3. Effects of the interaction between bio-fertilizers and Nano silica potassium will follow.

*Effects of bio-fertilizers*

Table 2 depicts the mean effects of the applied bio-fertilizers treatments as averaged across other variables ad the replicates on the total bacterial counts, total *A. lipoferum* counts, total *A. brasilense* counts, total mycorrhiza counts, dehydrogenase activity, phosphatase activity, and root colonization. In other words data in Table 2 tell about the biological situation in the root-zone under maize plants.

**TABLE 2. Effect of different bio fertilizers and their interactions on total bacterial and mycorrhizal populations, dehydrogenase activity, phosphatase activity, and root colonization of maize during 2016 and 2017 growth seasons under New Valley conditions**

| Char. / Bio fertilizer | Total bact. counts | Total *A. lipo.* counts | Total *A. braz.* counts | Total mycor. counts | Dehydro-genase activity | Phosphatase activity | Root coloization |
|---|---|---|---|---|---|---|---|
| **2016 Season** | | | | | | | |
| Control treatment | 14.24H | 12.25G | 7.81G | 0.77F | 3.74H | 1.17F | 45.09H |
| *Azos.. brazilienses.* | 20.63G | 15.33F | 13.65F | 1.17E | 5.53G | 1.74E | 50.15G |
| *Azos. lipoferum* | 25.72C | 19.70C | 17.83C | 1.90B | 6.90C | 1.86DE | 53.35F |
| Mycorrhiza | 22.54E | 16.83E | 14.73E | 1.49CD | 6.00E | 2.00CD | 60.77D |
| *Azos. bra.* + *Azos. lipo.* | 21.59F | 15.84F | 14.18EF | 1.41D | 5.72F | 1.80E | 54.90E |
| *Azos. bra.* + Mycor. | 23.59D | 18.58D | 16.00D | 1.66C | 6.51D | 2.05C | 62.48C |
| *Azos. lipo.* + Mycor. | 26.37B | 20.77B | 18.78B | 2.11B | 7.56B | 2.38B | 64.68B |
| Mixed of the three | 29.00A | 24.39A | 21.33A | 2.63A | 8.18A | 2.57A | 70.25A |
| **2017 Season** | | | | | | | |
| Control treatment | 19.93G | 14.83G | 9.90G | 0.84G | 4.21H | 1.32F | 50.13H |
| *Azos.. brazilienses* | 26.92F | 19.50F | 16.03F | 1.83F | 7.24G | 2.28E | 69.85G |
| *Azos. lipoferum* | 31.03C | 23.99C | 19.52C | 2.40C | 8.47C | 2.41DE | 71.61F |
| Mycorrhiza | 28.13E | 21.77E | 17.31E | 2.11E | 7.78E | 2.49D | 73.71C |
| *Azos. bra.* + *Azos. lipo.* | 27.28F | 21.65E | 17.14E | 1.94F | 7.40F | 2.32E | 72.36E |
| *Azos. bra.* + Mycor. | 29.48D | 23.18D | 18.55D | 2.24D | 8.15D | 2.56C | 73.12D |
| *Azos. lipo.* + Mycor. | 33.64B | 26.12B | 21.59B | 2.69B | 9.14B | 2.81B | 75.83B |
| Mixed of the three | 35.67A | 30.21A | 25.15A | 3v.07A | 9.78A | 3.07A | 79.59A |

*Total bacterial counts*

Data exhibited in Table 2 shows that all inoculation treatments could significantly establish higher bacterial counts in the rhizosphere of maize plants when compared to the control treatment in both seasons of study. *A. lipoferum* could significantly surpass mycorrhiza and *A. brasilense* in proliferating total bacterial count. *A. lipoferum* seems to suit the alkaline pH of the root-zone soil of the New Valley than *A. brasilense*, which prefers lower soil pH values. This is supported by the findings of Hungria et al. (2010) who recommended *Azospirillum brasilense* to be applied to maize as acidic peat-based inoculants. They found *A. lipoferum* promising, but not preferred to *A. brasilense*.

On the other side, mycorrhiza lives on symbiosis to get carbohydrates from the host plant. Therefore, mycorrhiza cannot be considered as a source of carbohydrates of the free living bacteria outside plant root, but rather secretes organic acids that goes out to the root-zone soil solution. These acids solubilize phosphorus from its resources especially mineral ones. Mycorrhiza can thus contribute to the proliferation of total microbial count in the free-living zone.

Inoculation with *A. brasilense* and *A. lipoferum* simultaneously proved to be significantly more effective than inoculation with *A. brasilense* alone, while it was less than *A. lipoferum* applied alone. It is clear that dropping the applied number of each of the two bacteria is to be held responsible for the obtained observation.

In case of mixed application of either *A. brasilense* or *A. lipoferum* with mycorrhiza, the applied number of each of the two bacteria is the same as that applied in the single application of both alone. This observation stands firmly behind the supremacy of the mixed application of *A. brasilense* with mycorrhiza as compared to applying each of them alone. The same held true for the mixed application of *A. lipoferum* with mycorrhiza. Comparing these two treatments that of applying *A. lipoferum* and *A. brasilense* together without inoculation with mycorrhiza; i.e. with initial soil mycorrhizal population, exposes the significant role of mycorrhiza in promoting the establishment of high total bacterial count.

Regarding the 3-microorganism bio-fertilizer, it can be said that despite the application of both bacteria at half their amount of sole application, the inclusion of mycorrhiza as a third bio-fertilizer could be highly promote the establishment of the highest significant total microbial count. Again, this observation assures the significant role played by mycorrhiza in promoting the establishment of high total bacterial count. All of the abovementioned observations held true in both years of study.

Total *A. lipoferum* counts and Total *A. brasilense* counts mean values depicted in Table 2 show approximately the same trends observed for the total bacterial count in Table 2. The highest significant total *Azospirillum lipoferum* counts were recorded with the 3-bio-fertilizer treatment. It is obvious that this combination treatment could proliferate the topmost promotion to the propagation of *Azospirillum lipoferum* in both years of study.

*Total mycorrhizal counts*

Total mycorrhizal counts mean values depicted in Table 2 show influences of the applied treatments on total mycorrhizal counts in the first year that were completely different from those observed in the second year of study. In the first year, bio fertilizers treatments were the only to show significant increases on total mycorrhizal counts. The control proliferated for the least total mycorrhizal counts, while the 3—bio-fertilizer treatment proliferated for the highest total mycorrhizal counts. An in-depth sight reveals that, when applied together, *A. lipoferum* was not significantly different from the treatment inoculated with both *A. lipoferum* and mycorrhiza together. Analogically, *A. brasilense* was not significantly different from the treatment inoculated with both *A. brasilense* and mycorrhiza together. In the second year of the trends' observed in Table 5 were, more or less, the same as those observed in Table 2. In 2017, bio-fertilizers showed to be significantly different, and so did the Nano—K and the interaction. The 3-bio-fertilizer treatment exhibited the topmost promotion to the propagation of mycorrhiza.

*Dehydrogenase activity*

Table 2 reveals that the trends observed on the total bacterial counts in the rhizosphere of maize roots were projected, approximately the same on dehydrogenase activity. This make sense because dehydrogenase activity comes mainly from the physiology practiced by the total bacterial count during their performance of metabolism. The highest significant dehydrogenase activity was

recorded with the combination treatment of applying half the recommended counts per ml of both *A. brasilense* and *A. lipoferum* together with mycorrhiza and sprayed with Nano—K fertilizer.

*Phosphatase enzyme*:

Phosphatase enzyme (Table 2) showed approximately the same trends observed with dehydrogenase activity. Both records reflect the trends in total bacterial counts in both seasons of study. The highest significant phosphatase enzyme was recorded with the combination treatment of applying half the recommended counts per ml of both *A. brasilense* and *A. lipoferum* together with mycorrhiza and sprayed with Nano—K fertilizer.

*Root colonization:*

Root colonization (Table 2) showed approximately the same trends observed with total bacterial counts in both seasons of study. The highest significant root colonization was recorded with the combination treatment of applying half the recommended counts per ml of both *A. brasilense* and *A. lipoferum* together with mycorrhiza and sprayed with Nano—K fertilizer.

*Effect of bio-fertilizers on maize plant height, grain yield, and HI*

Plant height (Table 3) shows a continuation of the effects observed in Table 2 in response to the applied bio-fertilizer treatments. In other words, the 3 different bio-fertilizer treatment led to the highest plant height as a translation to its superiority in proliferating the highest biological activities of nitrogen fixing bacteria and phosphate dissolving mycorrhiza (mycorrhiza does not dissolve phosphorus it just increased the P-uptake by plants through the increasing of absorbing area) in the rhizosphere of maize plant. This treatment showed the same significant superiority with grain yield and harvest index. This agrees with what Hungria et al. (2010) found of that using an inoculant containing bacteria that promote plant growth is likely to decrease costs of fertilizers, help with concerns over pollution and provide emphasis on sustainable agriculture. They recommended *Azospirillum* strains for maize (*Zea mays* L.) fertilization, which may increase grain yields by 24–30% in relation to non-inoculated controls.

In addition, Radwan & Nassar (2011) found that VA-mycorrhizal combined with bio-fertilizer cerealen and 105kg N/ha was the best treatment to obtain the highest growth and grain yield. Also, Mohammed (2012) showed that maize plants exhibited the highest significant plant height, grain yield and harvest index (HI) in response to phosphorus bio-fertilizer in the presence of different doses of nitrogen and $P_2O_5$.

With respect to grain content of N%, P%, K%, oil%, protein%, total carbohydrate%, and proline (µ mol/g DW), Table 4 reveals the effect of bio-fertilizers, mycorrhiza, and their interactions on these items in the dry matter during 2016 and 2017 growth seasons under New Valley conditions. It can be seen that the 3-bio-fertilizer treatment could show the highest significant accumulation of N, P, and K in grains when compared to other bio-fertilizer treatments in both years of study. It could also perform the highest significant production of oil, protein, and total carbohydrates, but the lowest proline accumulation in the dry matter of maize.

**TABLE 3. Effect of different bio fertilizers and their interactions on plant height, grain yield, and harvest index of maize during 2016 and 2017 growth seasons under New Valley conditions**

| Maize traits / Bio fertilizer | Plant height (cm) | Grain yield kg/fed. | Harvest index (%) | Plant height (cm) | Grain yield kg/fed. | Harvest index (%) |
|---|---|---|---|---|---|---|
| | 2016 Season | | | 2017 Season | | |
| Control treatment | 171F | 1918F | 34.64E | 175F | 1960F | 35.55G |
| *A. brazilienses* | 175E | 1970E | 35.10D | 179E | 2010E | 35.98F |
| *A. lipoferum* | 186B | 2077B | 36.03B | 189C | 2169B | 37.19B |
| Mycorrhiza | 180D | 2020D | 35.52C | 184D | 2065D | 36.58D |
| *A. bra.* + *A. lipo.* | 177E | 1987E | 35.20D | 182D | 2023E | 36.12E |
| *A. bra.* + Mycor. | 183C | 2045CD | 35.69C | 188C | 2122C | 36.82C |
| *A. lipo.* + Mycor. | 188AB | 2091B | 36.14B | 192B | 2202A | 37.27B |
| Mixed of the three | 190A | 2141A | 37.06A | 195A | 2242A | 37.91A |

**TABLE 4. Effect of 3 different bio fertilizers and their interactions on N%, P%, K%, oil%, Protein%, and total carbohydrates% of maize grains and proline content in the dry matter during 2016 and 2017 growth seasons under New Valley conditions**

| Char. / Bio fertilizer | N % | P % | K % | Oil % | Protein % | Total carbohydrate % | Proline (µ mol/g DW) |
|---|---|---|---|---|---|---|---|
| **2016 Season** | | | | | | | |
| Control | 1.24F | 0.70G | 0.68G | 3.38F | 8.72F | 68.25F | 8.27A |
| *A. braziliense* | 1.27EF | 0.73F | 0.71FG | 3.45E | 8.94EF | 69.44E | 7.91B |
| *A. lipoferum* | 1.45AB | 0.81E | 0.82BC | 3.63BC | 10.08AB | 70.20C | 7.26EF |
| Mycorrhiza | 1.31DE | 0.88C | 0.76DE | 3.54D | 9.16DE | 69.72D | 7.56CD |
| *A. bra.* + *A. lipo.* | 1.34D | 0.85D | 0.73EF | 3.50D | 9.38D | 69.63D | 7.73BC |
| *A. bra.* + Mycor. | 1.39C | 0.75F | 0.79CD | 3.60C | 9.70C | 70.07C | 7.38DE |
| *A. lipo.*+ Mycor. | 1.42BC | 0.95B | 0.84AB | 3.67B | 9.89BC | 70.41B | 7.15FG |
| Mixed | 1.48A | 1.01A | 0.87A | 3.74A | 10.24A | 71.58A | 7.02G |
| **2017 Season** | | | | | | | |
| Control | 1.25F | 0.73E | 0.75F | 3.42F | 8.85F | 69.32G | 8.58A |
| *A. braziliense* | 1.29E | 0.76DE | 0.78E | 3.48E | 9.07EF | 70.27F | 8.26B |
| *A. lipoferum* | 1.47AB | 0.84C | 0.88BC | 3.66BC | 10.21B | 71.13C | 7.72EF |
| Mycorrhiza | 1.32E | 0.91B | 0.83D | 3.58D | 9.29DE | 70.59E | 7.97CD |
| *A.bra.* + *A. lipo.* | 1.36D | 0.88BC | 0.81D | 3.52E | 9.54D | 70.45EF | 8.11BC |
| *A. bra.* + Mycor. | 1.41C | 0.78D | 0.86C | 3.62CD | 9.80C | 70.99D | 7.87DE |
| *A. lipo.*+Mycor. | 1.44BC | 0.99A | 0.90B | 3.70B | 10.02BC | 71.54B | 7.64F |
| Mixed | 1.49A | 1.03A | 0.93A | 3.77A | 10.49A | 72.67A | 7.53F |

The highest N accumulation could be referred to the highest proliferation of largest significant counts of bacteria under this treatment as was declared previously in the current research and consequently the fixation of the largest significant amounts of atmospheric N by the free-living *A. brasilense* and *A. lipoferum*.

The same treatment could show the highest significant P which can be attributed to the activity played by mycorrhiza which is a P releaser from its mineral or organic sources in the soil. For accumulating the highest significant K in grains owing to solubilizing effects of the bio-fertilizer treatments to K from various micaceous minerals in the soil, soil dressing of K, and/or K spraying with Nano silica, especially considering that the means values of the effects of bio-fertilizer treatments are basically averaged across the sprayed and unsprayed treatments and the replications.

With regard to the superiority of the same treatment in producing the highest significant oil, protein, and carbohydrates in the grains of maize, it can be referred to the highest carbon partitioning between the root and shoots of maize. This can be supported by what Zhang et al. (2013) emphasized the role played by Arbuscular mycorrhiza in controlling the fluxes of $CO_2$ between the aerial and underground parts of maize.

Concerning proline accumulation, the 3-bio-fertilizer treatment showed the least significant proline production owing to that the plants with this treatment were facing the least stresses in the ecological system (rhizosphere) or within the plants. This goes on the basis of the lower the stress, the lower the proline accumulation. This observation goes along with what was reported by Shamsul et al. (2012) who stated that a stressful environment results in an overproduction of proline in plants.

In conclusion, the combination treatment of inoculating maize grains with half the full dose of each of *A. lipoferum* and *A. brasilense* with mycorrhiza could significantly average the highest above all single and double bio-fertilizer treatments in accumulating N, P, and K in grains when compared to other bio-fertilizer treatments in both years of study. It could also

perform the highest significant production of oil, protein, and total carbohydrate, but the lowest proline accumulation in the dry matter of maize.

*Effect of nano silica K*

With regard to the effect of Nano silica K application, Table 5 clearly shows that using the Nano silica was steadily superior to non-spraying. This held true for all applied bio-fertilizers and the control, too. Nano silica contained potassium, which seems to be delivered to plant tissues with the rapid diffusion of Nano silica. This statistically significant observation points to an actual physical contribution of Nano silica K spray through dropping of spray solution onto the soil surface and reaching the soil solution. Therefore, it is believed that spraying procedure efficiency was supposed to be measured. This reasoning can be supported by the finding of Yuvakkumar et al. (2011) who mixed Nano-silica K powders with the soil and achieved vigorous root and vegetative growth of maize plants. Also, Amer & El- Emary (2018) reported that soil applied Nano-fertilizers can significantly improve soil productivity, enhance nutrients use efficiency, and protect plants from environmental stress. Spray solution dripping onto the soil surface can be understood to reach soil solution at the rhizosphere and contribute to the biological activities there. So, it is still needed to run more experimental work to emphasize the previous explanation. I addition, the efficiency of spraying process still also needs to be measured and verified.

It can be seen in Table 6 that spraying Nano-silica K was always superior to non-spraying in both years of study regarding all studied plant traits. The obtained results make sense where there is a direct physical access of potassium carried on and delivered by Nano-silica to aerial parts of maize plants. This observation goes along with that mentioned by Laane (2018) who stated that foliar sprays with silicates increases maize growth and yield. From another point of view, Guntzer et al. (2012) reported that silicon (Si) can mitigate environmental stresses and soil nutrient depletion and as a consequence is an alternative to the extensive use of phytosanitary and NPK fertilizers for maintaining sustainable agriculture. In other words, N, P, and K can be impregnated on Nano silica and sprayed on to the aerial parts of the plants. As so described, silicon is used as reservoir for N, P, and K when used as spray. In addition, Laane (2018) stated that foliar sprays with silicates increases vegetative growth and yield, while decreases biotic and abiotic stresses. He also added that despite being limited the available data on foliar Nano silica sprays show a tendency to decrease biotic stress and stimulate a limited increase in growth and yield.

**TABLE 5. Effect of nano silica spraying on chemical composition of maize during 2016 and 2017 growing seasons under New Valley condition**

| Char. / Nano silica spraying | Total bact. counts | Total *A. lipo.* counts | Total *A. braz.* counts | Total mycor. counts | Dehydr-ogenase activity | Phosph-atase activity | Root coloniz-ation |
|---|---|---|---|---|---|---|---|
| **2016 Season** | | | | | | | |
| Control | 20.59B | 15.35B | 13.99B | 1.37IB | 5.22B | 1.61B | 55.67B |
| Nano silica | 25.33A | 20.58A | 17.09A | 1.91A | 7.32A | 2.28A | 59.75A |
| **2017 Season** | | | | | | | |
| Control | 26.10B | 20.94B | 16.84B | 1.84B | 6.74B | 2.11B | 68.89B |
| Nano silica | 31.92A | 24.37A | 19.46A | 2.44A | 8.80A | 2.70A | 72.66A |

**TABLE 6. Effect of nano silica spraying on yield and its components of maize during 2016 and 2017 under the New Valley conditions**

| Trait / Nano silica K | Plant height (cm) | Grain yield (kg/fed.) | Harvest index (%) | Plant height (cm) | Grain yield (kg/fed.) | Harvest index (%) |
|---|---|---|---|---|---|---|
| | **2016** | | | **2017** | | |
| Control | 175B | 1947B | 34.86B | 179B | 2025B | 36.94B |
| Nano silica | 187A | 2115A | 36.48A | 192A | 2172A | 40.12A |

In conclusion, spraying Nano-silica K is eventually beneficial for the physiology taking place in the aerial parts of maize plants and indirectly in the rhizosphere.

With respect to grain content of N%, P%, K%, oil%, protein%, total carbohydrate%, and proline (µ mol/g DW), Table 7 reveals the effect of potassium Nano-silica spray on these items in the dry matter during 2016 and 2017 growth seasons under New Valley conditions. It can be observed that there is a straight forward superiority of spray on non-spray regarding accumulating N, P, K, oil, protein, and total carbohydrates, but the inverse regarding proline accumulation. Again, this makes sense owing to the direct connection with the physiology taking place in the aerial parts of maize plants. This observation goes along with that by Laane (2018) who stated that foliar sprays with silicates increase growth and yield.

*Effect of the interaction between bio-fertilizer and Nano-K*

Table 8 depicts the mean values of the effects of the interaction between bio-fertilizers treatments and spray with potassium Nano-silica averaged across the replicates on the total bacterial counts, total *A. lipoferum* counts, total *A. brasilense* counts, total mycorrhiza counts, dehydrogenase activity, phosphatase activity, and root colonization. The interaction between bio-fertilizers and spray with Nano silica K proved to be significant for plants receiving bio-fertilizers and sprayed with Nano than those not receiving Nano. This was evident for all measured biological activities in the rhizosphere, but for two activities; total *A. lipoferum* counts and mycorrhizal counts. The significance of those two interactions may have been mimicked by the vigorous effects of bio-fertilizers. Again, the compound treatment of the mixed three bio-fertilizers and spray with Nano silica K on the aerial parts of maize plants in both seasons of study.

Table 9 exhibits the effects of the interaction between bio fertilizers and spray with potassium Nano-silica on plant height, grain yield, and harvest index of maize during 2016 and 2017 under New Valley conditions. For maize plant height in table 9, the combination treatment composed of inoculation with half dose of both *A. brasilense* and *A. lipoferum* in addition to mycorrhiza at full dose with spraying the aerial parts of the plants with potassium Nano-silica proved to be superior to all other treatments in effecting for the highest significant maize plant height. This influence deems necessarily logic because plant height is a result of nutrition coming from the root system or from the shoots. Analogously, the same combination treatment could prove to be the topmost effective in producing the highest significant grain yield and harvest index in both years of study. All maize plant traits in table 9. The obtained results held true in both years of study.

With regard to the effect of the interaction between Nano silica spraying and bio fertilizers on the chemical composition of maize during 2016 and 2017 growing seasons under New Valley condition. Table 10 exhibits that the combination treatment composed of inoculation with half dose of both *A. brasilense* and *A. lipoferum* in addition to mycorrhiza at full dose with spraying the aerial parts of the plants with potassium Nano-silica proved to be superior to non-sprayed treatments in effecting for the highest significant accumulation of N, P, and K nutrients and the highest significant production of oil and protein, but insignificant in the accumulation of carbohydrates.

**TABLE 7. Effect of Nano silica spraying on chemical composition of maize during 2016 and 2017 growing seasons under New Valley condition**

| Char. / Nano silica spraying | N % | P % | K % | Oil % | Protein % | Total Carbo. % | Proline µmol/g DW |
|---|---|---|---|---|---|---|---|
| **2016 Season** | | | | | | | |
| Control | 1.26B | 0.69B | 0.72B | 3.47B | 9.35B | 68.37B | 8.41A |
| Nano silica | 1.46A | 0.98A | 0.83A | 3.65A | 9.67A | 71.45A | 6.67B |
| **2017 Season** | | | | | | | |
| Control | 1.27B | 0.73B | 0.78B | 3.50B | 9.49B | 69.61B | 8.93A |
| Nano silica | 1.48A | 1.01A | 0.89A | 3.68A | 9.83A | 72.13A | 6.99B |

**TABLE 8. Effect of the interaction between Nano silica spraying and bio fertilizers on chemical composition of maize during 2016 and 2017 growing seasons under New Valley condition**

| Nano silica X Bio / Char. | | Total bact. counts | Total *A. lipo.* counts | Total *A. braz.* counts | Total mycor. counts | Dehydro-genase activity | Phosph-atase activity | Root colonization |
|---|---|---|---|---|---|---|---|---|
| **2016 Season** | | | | | | | | |
| Without Nano silica | Cont. | 13.05j | 10.73 | 7.05k | 0.73 | 3.37o | 1.05h | 41.95m |
| | *A. braz.* | 18.63h | 12.35 | 11.93i | 0.92 | 4.72m | 1.48fg | 47.81l |
| | *A. lipo.* | 22.77e | 17.27 | 16.32de | 1.62 | 5.61i | 1.51fg | 50.58k |
| | Mycor. | 20.47g | 13.88 | 13.25h | 1.26 | 5.11k | 1.65f | 60.12f |
| | *A. braz.* + *A. lipo.* | 20.42g | 13.28 | 12.20i | 1.12 | 4.90l | 1.53f | 51.95j |
| | *A. braz.* + Mycor. | 21.55f | 15.60 | 14.82g | 1.35 | 5.38j | 1.69f | 61.72e |
| | *A. lipo.* + Mycor. | 23.23e | 18.30 | 17.03de | 1.75 | 6.09h | 1.91e | 62.83d |
| | Mixed | 24.60d | 21.35 | 19.33c | 2.22 | 6.55f | 2.06de | 68.38b |
| With Nano silica | Cont. | 15.43i | 13.77 | 8.57j | 0.81 | 4.11n | 1.29g | 48.23l |
| | *A. braz.* | 22.63e | 18.32 | 15.37fg | 1.42 | 6.35g | 1.99e | 52.48i |
| | *A. lipo.* | 28.67b | 22.13 | 19.33c | 2.18 | 8.19c | 2.22cd | 56.12h |
| | Mycor. | 24.62d | 19.77 | 16.22ef | 1.72 | 6.88e | 2.36c | 61.42e |
| | *A. braz.* + *A. lipo.* | 22.77e | 18.40 | 16.17ef | 1.70 | 6.54f | 2.06de | 57.85g |
| | *A. braz.* + Mycor. | 25.63c | 21.57 | 17.18d | 1.97 | 7.64d | 2.40c | 63.23d |
| | *A. lipo.* + Mycor. | 29.50b | 23.23 | 20.53b | 2.47 | 9.03b | 2.84b | 66.52c |
| | Mixed | 33.40a | 27.43 | 23.33a | 3.05 | 9.80a | 3.09a | 72.12a |
| **2017 Season** | | | | | | | | |
| Without Nano silica | Cont. | 17.38 | 13.63k | 10.32 | 0.78l | 3.81o | 1.19k | 46.15n |
| | *A. braz.* | 24.40 | 17.85i | 14.13 | 1.50k | 6.35m | 1.99i | 68.61l |
| | *A. lipo.* | 28.30 | 21.80f | 18.43 | 2.07h | 7.33i | 2.16ghi | 70.44k |
| | Mycor. | 25.55 | 20.17h | 15.83 | 1.74ij | 6.90k | 2.26fg | 73.33g |
| | *A. braz.* + *A. lipo.* | 24.70 | 20.58gh | 15.55 | 1.62jk | 6.54l | 2.06hi | 71.17j |
| | *A. braz.* + Mycor. | 27.35 | 20.90gh | 17.38 | 1.87i | 7.11j | 2.23fgh | 71.48i |
| | *A. lipo.* + Mycor. | 29.43 | 24.17d | 20.25 | 2.43ef | 7.64h | 2.40ef | 73.87f |
| | Mixed | 31.67 | 28.42b | 22.78 | 2.75c | 8.25f | 2.59de | 76.07c |
| With Nano silica | Cont. | 22.48 | 16.02j | 9.48 | 0.90l | 4.61n | 1.45j | 54.12m |
| | *A. braz.* | 29.43 | 21.15fg | 17.93 | 2.15gh | 8.14g | 2.56de | 71.10j |
| | *A. lipo.* | 33.77 | 26.18c | 20.60 | 2.73c | 9.61c | 2.65d | 72.78h |
| | Mycor. | 30.70 | 23.37e | 18.78 | 2.48de | 8.66e | 2.72cd | 74.08e |
| | *A. braz.* + *A. lipo.* | 29.85 | 22.72e | 18.73 | 2.27fg | 8.25f | 2.59d | 73.55g |
| | *A. braz.* + Mycor. | 31.62 | 25.47c | 19.72 | 2.62cd | 9.19d | 2.89c | 74.75d |
| | *A. lipo.* + Mycor. | 37.85 | 28.07b | 22.93 | 2.95b | 10.63b | 3.21b | 77.80b |
| | Mixed | 39.67 | 32.00a | 27.52 | 3.38a | 11.31a | 3.55a | 83.12a |

**TABLE 9. Effect of the interaction between Nano silica spraying and bio fertilizers on yield and its components of maize during 2016 and 2017 growing seasons under New Valley condition**

| Nano | Bio-fertilizer | Plant height (cm) | Grain yield kg/fed. | Harvest index (%) | Plant height (cm) | Grain yield kg/fed. | Harvest index (%) |
|---|---|---|---|---|---|---|---|
| | | | 2016 | | | 2017 | |
| without Nano silica | Cont. | 166o | 1834n | 33.83o | 168n | 1886o | 34.75o |
| | *A. braz.* | 169n | 1886m | 34.29n | 173m | 1936n | 35.18n |
| | *A. lipo.* | 180j | 1993i | 35.22j | 182i | 2095h | 36.39j |
| | Mycor. | 174l | 1936k | 34.71l | 177k | 1991l | 35.78l |
| | *A. braz.* + *A. lipo.* | 171m | 1903l | 34.39m | 175l | 1949m | 35.32m |
| | *A. braz.* + Mycor. | 177k | 1961j | 34.88k | 181j | 2048j | 36.02k |
| | *A. lipo* + Mycor. | 182h | 2007h | 35.33i | 185h | 2128g | 36.47i |
| | Mixed | 184f | 2057g | 36.25e | 188f | 2168e | 37.11f |
| With Nano silica | Cont. | 177k | 2002hi | 35.45h | 181j | 2033k | 36.34j |
| | *A. braz.* | 181i | 2054g | 35.91g | 186g | 2084i | 36.77h |
| | *A. lipo.* | 192c | 2161c | 36.84c | 196c | 2243c | 37.98c |
| | Mycor. | 186e | 2104e | 36.32e | 191e | 2139f | 37.37e |
| | *A. braz* + *A. lipo.* | 183g | 2071f | 36.00f | 188f | 2096h | 36.91g |
| | *A. braz.* + Mycor. | 189d | 2129d | 36.50d | 195d | 2196d | 37.61d |
| | *A. lipo* + Mycor. | 194b | 2175b | 36.95b | 199b | 2275b | 38.06b |
| | Mixed | 196a | 2225a | 37.86a | 202a | 2315a | 38.70a |

Also from table 10, this combination treatment produced the least amount of proline as compared with all other treatments and the control without or with potassium Nano-silica spray. This is accepted where proline accumulation takes place when plants suffer from adverse factors like drought and salt stress. It seems that the combination treatment has the plants not suffering from such stresses. This can stand a reason behind the production of the least amount of proline in the dry matter of maize plant. This finding stems from that observed by Shamsul et al. (2012) who reported that there was a positive correlation between proline accumulation and plant stress. They also stated that a stressful environment results in an overproduction of proline in plants. This explanation reveals that plants under the control treatment and all other applied treatments suffer from adverse effects at varying degrees relative to under the combination treatment.

## Conclusion

In conclusion, the combination treatment composed of inoculation with half dose of both *A. brasilense* and *A. lipoferum* in addition to mycorrhiza at full dose with spraying the aerial parts of the plants with potassium Nano-silica proved to be superior to all other treatments in effecting for the highest significant accumulation of N, P, and K nutrients and the highest significant production of oil and protein, but insignificant in the accumulation of carbohydrates. The same treatment produced the least proline in the dry matter of maize plants.

It can be seen that the applied treatments did not leave behind significantly different amounts of soil available P, soil available K, soil available N, soil carbon and calculated C/N ratio at harvest time in both years of study, except for significantly different amounts of soil available P and K, only in the first year. Again, the combination treatment mentioned above was the superior to all treatments in leaving behind the highest amounts of soil available P and K. albeit clear, it is essential to mention that the highest left behind P and K were found after the topmost absorption of nutrients and scoring the highest elemental accumulation in maize grains.

**TABLE 10. Effect of the interaction between Nano silica spraying and bio fertilizers on the chemical composition of maize during 2016 and 2017 growing seasons under New Valley condition.**

| Nano silica X Bio / Char. | | N % | P % | K % | Oil % | Protein % | Total carbohydrates % | Proline µ mol/g DW |
|---|---|---|---|---|---|---|---|---|
| **2016 Season** | | | | | | | | |
| Without Nano silica | Cont. | 1.14n | 0.55o | 0.63l | 3.29l | 8.55m | 66.71 | 9.14a |
| | *A. braz.* | 1.17m | 0.58n | 0.65k | 3.36k | 8.77l | 67.90 | 8.78b |
| | *A. lipo.* | 1.35gh | 0.66l | 0.76g | 3.54g | 9.91de | 68.66 | 8.13f |
| | Mycor. | 1.21l | 0.73j | 0.71i | 3.45i | 9.00jk | 68.18 | 8.43d |
| | *A. braz.* + *A. lipo.* | 1.24k | 0.70k | 0.68j | 3.41j | 9.22hi | 68.09 | 8.60c |
| | *A. braz.* + Mycor. | 1.29j | 0.60m | 0.74h | 3.51h | 9.53g | 68.53 | 8.25e |
| | *A. lipo* + Mycor. | 1.32i | 0.80i | 0.79f | 3.58f | 9.72f | 68.87 | 8.02g |
| | Mixed | 1.38ef | 0.86gh | 0.81e | 3.65e | 10.07c | 70.04 | 7.89h |
| With Nano silica | Cont. | 1.33hi | 0.85h | 0.73h | 3.47i | 8.88kl | 69.79 | 7.40i |
| | *A. braz.* | 1.37fg | 0.87g | 0.76g | 3.54g | 9.10ij | 70.98 | 7.04j |
| | *A. lipo.* | 1.55a | 0.96e | 0.87c | 3.72c | 10.24b | 71.74 | 6.39n |
| | Mycor. | 1.40e | 1.03c | 0.81e | 3.63e | 9.32h | 71.25 | 6.69l |
| | *A. braz.* + *A. lipo.* | 1.44d | 1.00d | 0.78f | 3.58f | 9.54g | 71.17 | 6.86k |
| | *A. braz.* + Mycor. | 1.49c | 0.90f | 0.84d | 3.69d | 9.86ef | 71.60 | 6.51m |
| | *A. lipo* + Mycor. | 1.52b | 1.10b | 0.89b | 3.76b | 10.05cd | 71.94 | 6.28o |
| | Mixed | 1.57a | 1.15a | 0.92a | 3.82a | 10.40a | 73.11 | 6.15p |
| **2017 Season** | | | | | | | | |
| Without Nano silica | Cont. | 1.15n | 0.59p | 0.69o | 3.33l | 8.68n | 68.06 | 9.55a |
| | *A. braz.* | 1.18m | 0.62o | 0.72n | 3.39k | 8.90m | 69.01 | 9.23b |
| | *A. lipo.* | 1.36h | 0.7m | 0.82j | 3.57g | 10.04de | 69.87 | 8.69f |
| | Mycor. | 1.22l | 0.77k | 0.77l | 3.49i | 9.12kl | 69.33 | 8.94d |
| | *A. braz.* + *A. lipo.* | 1.25k | 0.74l | 0.75m | 3.43j | 9.37ij | 69.19 | 9.08c |
| | *A. braz.* + Mycor. | 1.30j | 0.64n | 0.80k | 3.53h | 9.63h | 69.73 | 8.84e |
| | *A. lipo* + Mycor. | 1.33i | 0.85j | 0.84h | 3.61f | 9.85fg | 70.28 | 8.61g |
| | Mixed | 1.39g | 0.89h | 0.87f | 3.68e | 10.32bc | 71.41 | 8.50h |
| With Nano silica | Cont. | 1.35h | 0.87i | 0.80k | 3.51hi | 9.02lm | 70.58 | 7.61i |
| | *A. braz.* | 1.39g | 0.90g | 0.83i | 3.57g | 9.24jk | 71.53 | 7.29j |
| | *A. lipo.* | 1.57b | 0.98e | 0.93c | 3.75c | 10.38b | 72.39 | 6.75n |
| | Mycor. | 1.42f | 1.05c | 0.88e | 3.67e | 9.46i | 71.85 | 7.00l |
| | *A. braz.* + *A. lipo.* | 1.46e | 1.02d | 0.86g | 3.61f | 9.71gh | 71.71 | 7.14k |
| | *A. braz.* + Mycor. | 1.51d | 0.92f | 0.91d | 3.71d | 9.97ef | 72.25 | 6.90m |
| | *A. lipo* + Mycor. | 1.54c | 1.13b | 0.95b | 3.79b | 10.19cd | 72.80 | 6.67o |
| | Mixed | 1.59a | 1.17a | 0.98a | 3.86a | 10.66a | 73.93 | 6.56p |

**References**


Amer, M.M., El- Emary, F.A. (2018) Impact of foliar with Nano-silica in mitigation of salt stress on some soil properties, crop-water productivity and anatomical structure of maize and faba bean. *Env. Biodiv. Soil Security,* **2**, 25-38.

Becky, H., Martin, H., Joanna, H. (2001) How to optimize the drop plate method for enumerating bacteria. *J. Micro. Meth.* **44**, 121-129.

Bender, M.R., Wood, C.W. (2000) Total phosphorus in soil. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*, 45 Southern Cooperative Series Bull. No. 396.

Cappuccino, J.G, Sherman, N. (1992) Biochemical activities of microorganisms. In: "*Microbiology, A Laboratory Manual*". The Benjamin / Cummings Publishing Co. California, USA.

Faramawy, Fatma M.K. (2013) Aeroponic propagation of VA- mycorrhizal spores for soil inoculation as a bio-fertilizer. *Egypt. J. Phytopathol.* **41**(2), 145-157.

Ferreira, A.S., Pires, R.R., Rabelo, P.G., Oliveira, R.C., Luz, J.M.Q., Brito, C.H. (2013) Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. *Applied Soil Ecology,* **72**, 103-108.

Fulchieri, Mónica, Lucangeli, C., Bottini, R. (1993) Inoculation with *Azospirillum lipoferum* affects growth and bibberellin status of corn seedling roots. *Plant and Cell Physiology*, **34**(8), 1305–1309.

Gerdemann, J.W., Nicolson, T.H. (1963) Spores of mycorrhizal endogone species extracted from spil by wet-sieving and decanting. *Transactions of British Mycological Society*, **46**(2), 235-244.

Giovannetti, M., Mosse, B. (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist,* **84**, 489–500.

Guntzer, F., Keller, Catherine, Meunier, J-D. (2012) Benefits of plant silicon for crops: A review. *Agronomy for Sustainable Development*, **32**, 201-213.

Hungria, Mariangela, Campo, R.J. , Emanuel, Souza M., Pedrosa, F.O. (2010) Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil*, **331**, 413–425.

Jackson, M.L. (1973) "*Soil Chemical Analysis*", pp. 38-56. Printice Hall, Inc., Englewood Cliffs, N.J. Library of Congress, USA.

Knudsen, D., Peterson, G.A., Pratt, P.F. (1982) Lithium, sodium, and potassium. In: "*Methods of Soil Analysis*. *Part 2. Chemical and Microbiological Properties*", A.L. Page, R.H. Miller, D.R. Keeney (Eds.), 2nd edn. pp. 225–45. Agronomy Monograph No. 9 (ASA/SSSA: Madison, WI, USA).

Krieg, N.R., Holt, J.G. (1984) "*Bergey's Manual of Systematic Bacteriology*". Gram positive Bacillus. V01. 1, Section 4, cited from, p. 220–229, Williams and Wilkins, Baltimore,*USA*.

Laane, H-M. (2018) The effects of foliar sprays with different silicon compounds. *Plants*, **7**(2), 45. https://doi.org/10.3390/plants7020045

Machado, Cynthia Torres deT., Furlani, Ângela Maria C. (2004) Root phosphatase activity, plant growth and phosphorus accumulation of maize genotypes. *Scientia Agricola*, **61**(2), 216-223.

Mohammed, A.A. (2012) Effect of bio-fertilizer on physiology of growth and development of maize (*Zea mays* L.) in Sulaimani region. Mesopotamia *J. Agric*. **40**(1), 9–21.

Morais, T.P., Brito, C.H., Brandão, A.M., Rezende, W.S. (2016) Inoculation of maize with *Azospirillum brasilense* in the seed furrow. *Revista Ciencia Agronomica*, **47**(2). http://dx.doi.org/10.5935/1806-6690.20160034

Muthukumar, T. (1017) Arbuscular mycorrhizal fungus influence maize root growth and architecture in rock phosphate amended tropical soil. *Anales de Biología*, **39**, 211-222.

Olsen, S.R., Cole, C.J., Watanabe, F.S., Dean, L.A. (1982) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. No. 939, USDA. U.S. Government Printing Office, Washington, DC.

Page, A.L., Miller, R.H., Keeney, D.R. (1982) "*Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*". 2nd ed., Madison, Wisconson, U.S.A.

Peach, K., Tracey, M.V. (1956) Modern methods of combination of pig slurry and mineral nitrogen fertilizer. *J. Agric. Sci. Camb.* **127**, 151 -159.

Phillips, J.M., Hayman, D.S. (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, **55**, 158–161.

Pious, T., Aparna, C., Sekhar, R., Upreti, M.M., Sadiq, S.P. (2015) Optimization of single plate serial dilution spotting (SP-SDS) with sample anchoring as an assured method for bacterial and yeast cfu enumeration and single colony isolation from diverse samples. *Biotechnology Reports*, **8**, 45–55.

Pramer, D., Schmidt, E.L. (1964) "*Experimental Soil Microbiology*". Burgess Publ. Co., Minnesota, USA.

Radwan, F.I., Nassar, M.A.A. (2011) Response of maize hybrid to biofertilization, soil nitrogen application and weed control. Alexandria Science Exchange Journal, **32**(4), 409-421.

Rossel, D., Tarradellas, J. (1991) Dehydrogenase activity of soil microflora: Significance in ecotoxicological tests. *Journal of Environmental Toxicology and Water Quality*. **6**(1), 17-33.

Salgado, F.H.M., Moreira, F.M. deS., Siqueira, J.O., Barbosa, R.H., Paulino, H.B., Carneiro, M.A.C. (2017) Arbuscular mycorrhizal fungi and colonization stimulant in cotton and maize. *Ciência Rural, Santa Maria,* **47**(6), e20151535. http://dx.doi.org/10.1590/0103-8478cr20151535

Shekh, B.A. (2006) Biotechnology and biofertilization: Key to sustainable agriculture. Scientific Issue, (1) Das, K., R.Dang, T. N.

Shamsul, H., Qaiser, H., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A. (2012) Role of proline under changing environments: A review. *Plant Signaling & Behavior,* **7**(11), 1456–1466.

SPSS (2014) https://www.ibm.com/analytics/spss-statistics-software.

Tabatabai, M.A., Bremmer, J.M. (1969) Use of p-nitrophenylphosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1**, 301-307.

Thalmann, A. (1967) Uber die microbiello Akivitat undihr Beziehung Zu fruchtbartkeits merkmalen eini-ger Acherboden unter besonderer Berucksi chtigung der dehydrogenase akativitat (TTC. Redukation). Biss Gieben *PH.D. Thesis*, W. Germany.

Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, D. (1986) Mesure de taux de mycorrhization VA d'un systeme radiculaire recherché de methodes d'estimation ayant une signification fonctionnelle. In: "*Physiological and General Aspects of Mycorrhizae*". V. Gianinazzi-Pearson and S. Gianinazzi (Eds.), pp. 217-221. INRA Publications, Paris.

Walkley, A., Black, I.A. (1984) An examination of Degtjareff Method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **37**, 29–37.

Yuvakkumar, R., Elango, V., Rajendran, V., Kannan, N.S., Prabu, P. (2011) Influence of Nano-silica powder on the growth of Maize Crop (*Zea mays* L.). *International Journal of Green Nanotechnology,* **3**(3), 180-190.

Zhang, X.B., Xu, M.G., Sun, N., Wang, X.J., Wu, L., Wang, B.R., Li, D.C. (2013) How do environmental factors and different fertilizer strategies affect soil $CO_2$ emission and carbon sequestration in the upland soils of southern China? *Applied Soil Ecology*, **72**, 109-118.

## تأثير التسميد الحيوى والنانو سيليكا على نبات الذرة بالوادى الجديد

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أقيمت تجربة حقلية بالوادي الجديد لدراسة مدى استجابة نبات الذرة لثلاثة أنواع من الأسمدة الحيوية وهي: الآزوسبيريلليم برازيلينس و الآزوسبيريلليم ليبوفيروم والميكورهيزا بالإضافة إلى ثلاثة تفاعلات حيوية ثنائية وتفاعل حيوي ثلاثي واحد وذلك مقابل معاملة الكنترول (بدون تسميد حيوي). كل المعاملات الثمانية نفذت إما بمفردها أو مع الرش بسيليكات البوتاسيوم النانونية. وقد نفذت ال 16 معاملة مركبة في تصميم القطع المنشقة مرة واحدة في ثلاثة قطاعات كاملة عشوائية. والهدف الرئيسي من البحث الحالي هو أن نفحص التأثيرات المتصاحبة لكل المعاملات المطبقة على بعض القياسات الحيوية للذرة. وقد أظهرت النتائج المتحصل عليها أن المعاملة المركبة المكونة من تلقيح تقاوي الذرة بنصف العدد الموصى به لكل من الآزوسبيريلليم برازيلينس والآزوسبيريلليم ليبوفيروم مع الميكورهيزا والرش بسيليكات البوتاسيوم النانونية مهدت معنوياً لأعلى عدد كلي من البكتيريا، والآزوسبيريلليم برازيلينس والآزوسبيريلليم ليبوفيروم والميكورهيزا، وكذلك أعلى نشاط لإنزيم الديهيدروجينيز، وأنزيم الفوسفاتيز، وأعلى غزو للجذور بالميكورهيزا خلال موسمي الدراسة الحالية. وقد أظهر الآزوسبيريلليم ليبوفيروم أداءً معنويا أفضل من الآزوسبيريلليم برازيلينس. وقد أدى الرش بسيليكات البوتاسيوم النانونية إلى تأثير معنوي غير مباشر على أعداد الكائنات الدقيقة في منطقة انتشار الجذور لنبات الذرة في موسمي الدراسة. وينصح بإضافة سيليكات البوتاسيوم النانونية عن طريق التربة في منطقة انتشار الجذور لتمارس دورها مباشرة في انعاش الكائنات الدقيقة بالتربة.