Effect of Rock Phosphate, Herbicides Particles and Phosphate Solubilizing Bacteria on Growth and Chemical Composition of Corn Plants Grown in Calcareous Soil

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ABSTRACT: Pots experiments were conducted on corn plants (*Zea mays*, L.), Trihybrid 302, at the experimental greenhouse of the Agriculture Faculty (Saba-Basha), Alexandria University, Egypt to evaluate the effect of rock phosphate, some nano-herbicides particles and phosphate solubilizing bacteria on growth and chemical composition of corn plants grows in calcareous soil. The experiment was designed as spilt -spilt plot design with four replicates. Each replicate contained 8 treatments as follows: main plot as rock phosphate (0, 50 and 100), subplot as herbicides (Control, herbicide, nano-herbicide), sub-sub plot phosphate solubilizing bacteria (Uninoculated, *Bacillus megaterium*). Results indicated that, the interaction with rock phosphate at a rate 100% of the recomonded dose and nano-herbicides in the case of plants inoculated with *B. megaterium* bacteria recorded the maximum mean values of vegetative growth of maize (plant height, total chlorophyll (SPAD), shoot fresh weight, shoot dry weight, leaf area index, as compared of control plants which gave the minimum mean values of these characters. Also, rock phosphate at 100% rate, nano-herbicides and bacteria gave the highest mean values of root fresh and dry weight, as compared with control. Moreover, rock phosphate at 100% rate, nano-herbicides and bacteria gave the highest mean values of N, P, K percentages in shoot, as compared with control.

Keywords: *Bacillus megaterium*, Nano-herbicids, phosphate solubilizing bacteria , Rock phosphate (RP).

INTRODUCTION

Phosphorus (P) is one of the key components required for plant development and growth; it makes up about 0.2% of the dry weight of the plant. It is the major element second among the mineral nutrients most widely used to limit crop development (Azziz *et al.,* 2012) and plays a vital role in plant productivity and metabolism by providing the energy needed for metabolic mechanisms (Elser *et al.,* 2007) The majority of soils like arid and semiarid regions are deficient in available phosphorus (Memon *et al.*, 1992, Kang *et al.,* 2011). The availability of P is affected by soil chemical properties and human management activities.

Inorganic P is mined to produce chemical P fertilizers that are extensively applied to cropland (Elser and Bennett, 2011; Penuelas *et al.,* 2013). The majority of soluble inorganic P, however, is rapidly immobilized by soil fixation and becomes unavailable for plant uptake, leading to low P-use efficiency (Kochian, 2012). Soil P must thus be managed to increase its use efficiency.

Rock Phosphate is a particular word that describes naturally occurring mineral assemblies comprising an elevated concentration of phosphate minerals (Zapata and Roy, 2004). Rock phosphates are generally apatite, containing varying percentages of P_20_5 in a calcium matrix, and either directly applied to the soil, in particular in organic farming or in the production of water-soluble phosphorus fertilizers. Sedimentary rock phosphates are reported to be appropriate for direct use as fertilizers only under certain environmental conditions **(**Chien and Friesen, 2000 and Zapata and Roy, 2004). Microbial production of organic acids and acid phosphatase has important role in mineralizing organic P present in soil (Cherr *et al*., 2006; Wilhelm *et al.,* 2007).

Phosphate-solubilising bacteria (PSB) inoculants have been assayed but their effectiveness in the soil-plant system is still unclear. In addition, the role of the inoculated PSB that supplies P to the plant seems limited because the transient nature of the compounds released by PSB responsible for phosphate solubilization, and because the possible re-fixation of phosphate ions on their way to the root surface, if any solubilization does take place (Barea *et al*., 2007). Many researchers proved that PSB plays a key role in soil organic P (P*o*) transformations (Frossard *et al.,* 1995) through excretion of phosphatase enzymes (Eichler *et al.,* 2004), mineralization of P from organic sources (Gressel and McColl, 1997), and also synthesis and release of P*o* (Oberson *et al.,* 2001). Besides, microorganisms can solubilize sparingly soluble P*i* forms (Iyamuremye *et al*., 1996). Phosphate solubilizing bacteria (PSB) are a group of these microorganisms that can transform insoluble P compounds into available forms by secreting organic acids, and they may be used as inoculants to enhance P availability for plants (Khan *et al.,* 2007). Also, they can promote plant growth via producing hormones, such as cytokinin and indole acetic acid (Coutinho *et al.,* 2012, Zak *et al.,* 2018). Despite some PSB present in plant rhizospheres and soil, the amount of P released by these microorganisms is usually insufficient to meet the demand of growing plants (Collavino *et al.,* 2010). High-efficiency PSB has the potential for making a great contribution to the decrease of environmental pollution and promoting ecological balance by replacing chemical fertilizers (Zak *et al.,* 2018). Consequently, there is an urgent need to investigate the effects of selected high-efficiency PSB on plant nutrition and growth.

During the application of herbicides, a large portion of these chemical accumulates in the top layer soil (0-15 cm) where most of the microbiological activities occur (Das and Kole, 2006). Microorganisms degrade a variety of carbonaceous substances including the accumulated herbicides in the soil to derive their energy and other nutrients for their cellular metabolism (Das *et al.,* 2003). As a result, the number of microbial biomass increases which favorably influences the transformations of plant nutrients in the soil (Das *et al.,* 2003). Reports are also available (Selvamani and Sankaran, 1993; El-Ghamry *et al.,* 2001) on the deleterious effect of herbicides on growth and activities of microorganisms in the soil. Moreover, the interaction between the herbicides and microorganisms vary depending upon the type of herbicides and microorganisms (Nongthombam *et al.,* 2008).

Current pest management relies heavily on the application of pesticides, such as insecticides, fungicides, and herbicides. In spite of many advantages, like high availability, fast action, and reliability, pesticides have harmful side effects towards non-target organisms, the resurgence of the pest population, and the development of resistance (Stephenson, 2003). Furthermore, it is estimated that 90% of applied pesticides are lost during or after application (Stephenson, 2003; Sinha *et al.,* 2017). As a result, there is an increased motivation to develop cost-efficient, high-performing pesticides that are less harmful to the environment.

Nanotechnology has led to the development of new concepts and agricultural products with immense potential to manage the aforementioned problems. Nanotechnology has substantially advanced in medicine and pharmacology but has received comparatively less interest for agricultural applications (Sinha *et al.,* 2017; Balaure *et al.,* 2017). The use of nanotechnology in agriculture is currently being explored in plant hormone delivery, seed germination, water management, transfer of target genes, nanobarcoding, nanosensors, and controlled release of agrichemicals (Hayles *et al.,* 2017).

Material scientists have engineered nanoparticles with desired characteristics, like shape, pore size and surface properties. So that nanoparticles can then be used as protectants or for precise and targeted delivery via adsorption, encapsulation, and/or conjugation of an active, such as a pesticide (Khandelwal *et al.,* 2016). As agricultural nanotechnology develops, the potential to provide a new generation of pesticides and other actives for plant disease management will greatly increase. The use of nanoparticles to protect plants can occur via two different mechanisms: (a) nanoparticles themselves providing crop protection, or (b) nanoparticles as carriers for existing pesticides or other actives, such as double-stranded RNA (dsRNA), and can be applied by spray application or drenching/soaking onto seeds, foliar tissue, or roots. Nanoparticles, as carriers, can provide several benefits, like (i) enhanced shelf-life, (ii) improved the solubility of poorly water-soluble pesticides, (iii) reduced toxicity, and (iv) boosting site-specific uptake into the target pest (Hayles *et al.,* 2017). The objective of this study was to determine the effect of rock phosphate, some nano-herbicides particles and phosphate solubilizing bacteria on growth and chemical composition of corn plants grows in calcareous soil.

MATERIALS AND METHODS

This work was carried at the greenhouse of Agricultural Faculty (Saba Basha), Alexandria, Egypt during marc 2019 growing season.

Soil physicochemical characteristics

Surface calcareous soil sample (0-15 cm) was collected from Burg Al Arab Alexandria - Egypt. The sample was air-dried, ground to pass into 2 mm sieve and thoroughly mixed before using. The characteristics of this soil are presented in Table (1).

The methods used for soil analyses were those described by Page *et al.* (1982).

Table (1). Some Initial soil physical and chemical properties of the experimental soil

Properties	Values
Mechanical analysis (%)	
Clay	16.48
Silt	16.00
Sand	67.52
Soil texture	Sandy loam
pH (1:2 water suspension)	8.4
EC dsm^{-1} (1:1 water extract)	4.0
Total $CaCO_3$ (%)	3.51
Organic matter (%)	3.01
Soluble Cations (meq/l)	
Calcium	8.5 meq/l
Magnesium	7.2 meq/l
Sodium	13.04 meq/l
Potassium	1.28 meq/l
Soluble Anions (mg/l)	
Bicarbonate	1.20
Chloride	28.05
Sulphate	5.37
Total Phosphorus (mg/ kg soil)	42.5
Available P (mg/ kg soil)	36.5

Rock phosphate (RP)

Low- grade rock phosphate sample from a sedimentary phosphate rock deposit supplied in a fine powder to pass through a 400- mesh standard sieve by Al Ahram mining and natural fertilizer Company in Egypt.

Herbicides

The glyphosate-based herbicide Roundup (N-(phosphonomethyl) glycine) was used in two forms, the regular form (4g/L) and nanoparticle.

Preparation of nano- glyphosate-based herbicide Roundup

The nano form of the herbicide was carried out at City of Scientific Research and Technology Applications (SRTA-City). Small quantity of conventical herbicide powders were introduced into the ball milling for further grinding to reach the nano scale of the herbicide. Glyphosate-based herbicide Roundup was undergone and ground using a planetary ball mill (Gj-LC 3210CH). The mixed powders together with grinding balls were put into the milling jar with the weight ratio of ball-to-powder of 20:1. Argon was adopted as protective atmosphere of the milling jar. The total milling time was set as 20 h with each 30 min resting after 30 min running to prevent overheating. The particles size of the samples used for performance test was strictly limited between 48 and 72 mm. Figure (1) illusterated (SEM) of nano herbicide showing the nano shape and size at 25000X.

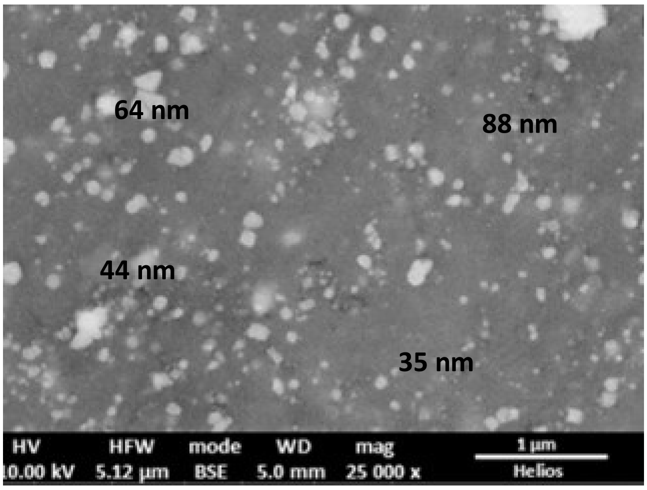

Figure (1). Scanning electron microscopy (SEM) of nano glyphosate showing the nano shape and size at 25000X.

Scanning electron microscopic studies

Surface morphology of nano- particles was of glyphosate confirmed by scanning electron microscopy (SEM). Analyses of nano- particles were performed using an H-7500 scanning electron microscope (Hitachi, Japan) with an acceleration voltage of 80 kV. Nano- particles were imaged after airdrying a droplet on a carbon-coated 200-mesh copper grid. The suspension containing nano- particles were samped by SEM analysis using (JEOL-SEM 100CX) at the electron microscope Unit at City of Scientific Research and Technology Applications (SRTA-City) according to the method described by (Elgorban *et al.,* 2016).

Bacteria inoculum

Bacillus megaterium, strain 1062, was obtained from the Bio-fertilization unit, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The bacteria were reproduced on Luria Bertani (LB) medium comprising of (g/L) tryptone, 10; yeast extract, 5; NaCl, 5. The pH of the medium was adjusted to 7.2–7.4 using 1 N HCl or 1 N NaOH and sterilized by autoclaving at 121 °C for 15 minutes. The culture was maintained at 30 °C for 3 days. After seeds germination, bacterial suspension was added to each pot as 15 ml (1.3×10^6 viable cells/ml).

Cultivar

Corn cultivar, Trihybrid 302, was used in this study. The seeds of this cultivar were obtained from the Agricultural Research Center in Giza, Ministry of Agriculture and Land Reclamation, Egypt.

Pot experiment

A pot experiment was carried out in the greenhouse at the Faculty of Agriculture (Saba Basha), Alexandria University, Egypt uses the enriched soils with rock phosphate. Plastic pots (11.5 cm deep and 17 cm in diameter) with holes in their bottom) were filled with 2.0 kg of the enriched soils with three levels of rock phosphate (0, 50 and 100% of recommended dose) and soil with rock (RP) was mixed at the rate of 9,2 g/kg soil as recommended. Seeds of corn Trihybrid 302 cultivar were treated by 0.05 % NaOC1 solution subsequently washed several times with distilled water and planted in each pot.

The experiment was carried out in 3 factors as split – split-plot design with three levels of rock phosphorus ($RP_0\%$, $RP_{50\%}$, and $RP_{100\%}$) as the main plot. The herbicide treatments {control (HP), normal pesticides (HP1), nano-pesticides (HP2) } were randomly distributed in the subplot, While the phosphate solubilizing bacterial treatments {control (Un-Inoc)*,* *Bacillus megaterium* (Inoc)} were arranged in the sub – subplots. The treatments were replicated 4 times Four seeds of corn were planted in each pot and after two weeks, the plants thinned to two plants after emergence. Herbicide were added as (Roundup). The N fertilizer was applied in three equal does through the growth period at the rate of 50 mg/20 ml water for each pot after cultivation. The P, K, and Mg fertilizers were applied before filling the pots with soil. All pots were irrigated with tap water every day to keep the soil at 70% of its field capacity by the regular weighting of pots (Figure 2).

Data recorded

Vegetative parameters

Two plants per pot were taken to determine the plant height (cm), fresh weight (g/plant). Two plants were air-dried until constant weight and the average dry weight of root and shoot (g/plant) was calculated.

- **Plant height (cm/plant).**

The average value of plant height was randomly measured per plant in cm.

- **Leaf area index.**

The LAI was calculated according to Watson (1952) as follows:

$$\text{LAI}= \frac{leaf\ area\ /\ plant}{plant\ ground\ area}$$

- **Fresh weight /plant (g).**

Plant fresh weight (leaves and stem) was randomly calculated per plant (g).

- **Root and Shoot dry weight/plant (g).**

The plants per pot were air-dried until constant weight. The average weight was calculated for each sample (g).

Plant analysis

Plant samples were taken from each pot, at a suitable age washed with running tap water, and then with distilled water. The samples were air-dried, milled and stored for analysis. 0.5g of plant powder was wet-digested with H_2SO_4–H_2O_2 mixture (Lowther, 1980) and the following determinations were carried out in the digested solution.

Nitrogen content

Total nitrogen was determined in digested plant material colorimetrically by Nessler's method (Chapman and Pratt, 1978) using 1ml of Nessler solution (35g IK/100 ml distilled water + 20g $HgCl_2$/500 ml d.w) +120g NaOH/250 ml d.w. Reading was achieved using wavelength of 420 nm.

$$\%\text{N} = \text{NH4} \times 0.776485$$

Phosphorus content

Total phosphorus in wet ash was determined in the plants by the Vanadomolybdate yellow methodas described by Jackson (1967). Measurement was done using Milton Roy spectronic 21D spectrophotometer.

Potassium content

Total potassium content was determined according to the method described by Jackson (1967) using Beckman flame photometer.

Concentration index

Plants were collected after 61 days from planting. Before the harvest time, the Chlorophyll concentration index (CCI) was measured using the chlorophyll meter (Model: CCM-200).

Bacterial media preparation for total count bacteria (cfu)

According to the literature, the Pikovskaya's agar medium (PVK) was found to be as selective media for the isolation of PSM. The pH value was maintained at 7. PSM was isolated from each soil sample by serial dilution and spread plate method. One gram (1g) of soil sample was dispersed in 9 ml of autoclaved distilled water and thoroughly shaken. 1 ml of the above solution was again transferred to 9ml of sterile distilled water to form 10^{-2} dilution. Similarly 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} serials were made for each soil sample. 0.1ml of each dilution was spread on Pikovskaya's agar medium (PVK) containing insoluble tricalcium phosphate and incubated at 27 - 300C for 7 days. Colonies were counted within the dilution which gave 30-300 colonies.

Statistical analysis

The collected data were arranged in a randomized complete block design and replicated four times. Data were statistically analyzed for ANOVA and means comparison to fulfill the significance according to Steel and Torrie (1982). A significance level of α = 0.05 was used in all analysis.

RESULTS AND DISCUSSION

A) Effect of rock phosphate, herbicides and phosphate solubilizing bacteria on vegetative growth

Results presented in Table (2) show the effects of different concentrations of rock phosphate, phosphate solubilizing bacteria, and herbicides on plant height, stem fresh weight (g), stem dry weight (g) and leaf area index. Concerning the effects of rock phosphate, data indicated that increasing rock phosphate concentrations increased plant height, stem fresh weight (g), stem dry weight (g) and leaf area index (LAI), as compared with control treatment. Generally, rock phosphate at 100% rate gave the higher plant height (77.90), stem fresh weight (202.26 g), stem dry weight (40.45 g) and leaf area index (3.88), as compared with control treatment which gave the lowest values of plant height (51.26cm), stem fresh weight (129.44 g), stem dry weight (25.88 g) and leaf area index (2.48), respectively. Also, the data in Table (2) indicated that nano-herbicides recorded the maximum values of plant height (78.02 cm), shoot fresh weight (200.12 g), shoot dry weight (40.02 g) and leaf area index (3.84), as compared with control treatment which gave the minimum values of plant height (50.75 cm), shoot fresh weight (131.30 g), shoot dry weight (26.26 g) and leaf area index (2.52).

On the other hand, inoculation with phosphate solubilizing bacteria recorded the highest mean values of plant height (67.37 cm), shoot fresh weight (173.16 g), shoot dry weight (34.61 g) and leaf area index (3.32), as compared with control treatment which gave the minimum values of plant height (60.25 cm), shoot fresh weight (155.85 g), shoot dry weight (31.17 g) and leaf area index (2.99), respectively.

The interaction between rock phosphate and herbicides was high significantly on plant height, stem fresh weight, stem dry weight, and leaf area index, also, the interaction between rock phosphate and phosphate solubilizing bacteria (*B. megaterium*) was high significantly on plant height, shoot fresh weight, shoot dry weight, and leaf area index. The interaction between herbicides and bacteria was high significantly on plant height, shoot fresh weight, shoot dry weight, and leaf area index, but, the interaction between rock phosphate, herbicides and *B. megaterium* bacteria showed insignificant effect on plant height, shoot fresh weight, shoot dry weight, and leaf area index. Our results are in agreement with those of Han and Lee (2005), who also reported that plant height improves with PSB inoculation over 27%. PSB inoculation improves the availability of minerals content and nutrients which promotes plant growth, increases phosphorus uptake and photosynthesis.

Table (2). Mean effects of rock phosphate, herbicides and phosphate solubilizing bacteria on vegetative growth

Treatments	Plant height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf area index (LAI)
A) Rock phosphate				
Control	51.26	129.44	25.88	2.48
50%	62.27	161.81	32.33	3.11
100%	77.90	202.26	40.45	3.88
LSD(0.05)	**2.97**	**0.82**	**0.17**	**0.03**
B) Herbicides				
Control (HP)	50.75	131.30	26.26	2.52
Herbicide(H1)	62.65	162.10	32.39	3.11
Nano-herbicide (H2)	78.02	200.12	40.02	3.84
LSD (0.05)	**0.88**	**0.39**	**0.09**	**0.01**
C) PSB				
Un-inoculated	60.25	155.85	31.17	2.99
Inoculated	67.37	173.16	34.61	3.32
LSD (0.05)	**0.53**	**0.08**	**0.04**	**0.003**
Interaction				
A× B	**	**	**	**
A×C	**	**	**	**
B×C	**	**	**	**
A× B × C	**Ns**	**	**	**

(Asterisks indicate a significant difference compared to the respective non-inoculated control, according to Tukey's HSD test)

Perusal data presented in Table (3) revealed that rock phosphate at 100% rate treatment recorded the maximum root fresh weight (92.54 g) and root dry weight (18.50 g), as compared with control treatment which gave the minimum root fresh weight (59.22 g) and root dry weight (11.84 g), respectively. It is clear from the date in Table (3) that nano-herbicides significantly increased root fresh weight (91.56 g) and root dry weight (18.31g), as compared with control treatment, which gave the lowest mean values of root fresh weight (60.15 g) and root dry weight (12.01 g).

Moreover, the inoculation with phosphate solubilizing bacteria recorded the highest mean values of root fresh weight (79.23 g) and root dry weight (15.84g), as compared with control treatment which gave the minimum values of root fresh weight (71.36 g) and root dry weight (14.26 g), succesively.

The interaction between rock phosphate and herbicides were high significant on root fresh weight and root dry weight. Rock phosphate and phosphate solubilizing bacteria, herbicides and phosphate solubilizing bacteria, rock phosphate, herbicides, and phosphate solubilizing bacteria.

Table (3). Mean effects of rock phosphate treatments, herbicides and phosphate solubilizing bacteria (*Bacillus megaterium*) on root fresh and dry weight

Treatments	Root fresh weight (g)	Root dry weight (g)
A) Rock phosphate		
Control	59.22	11.84
50%	74.11	14.80
100%	92.54	18.50
LSD (0.05)	**0.78**	**0.15**
B) Herbicides		
Control (H1)	60.15	12.01
Herbicide (H2)	74.16	14.83
Nano-herbicide (H3)	91.56	18.31
LSD (0.05)	**0.43**	**0.07**
PSB(*Bacillus megaterium*)		
Un-inoculated	71.36	14.26
Inoculated	79.23	15.84
LSD(0.05)	**0.15**	**0.15**
Interaction		
A×B	**	**
A×C	**	**
B×C	**	**
AxBxC	**	**

(Asterisks indicate a significant difference compared to the respective non-inoculated control, according to Tukey’s HSD test)

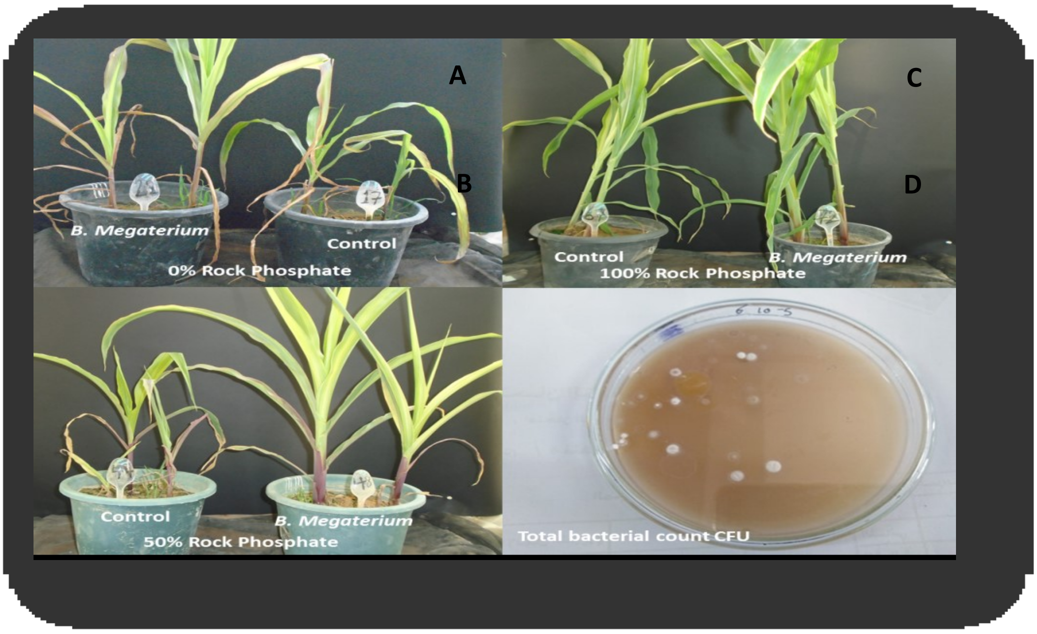

Figure (2). Schematic diagram of **(A)** Representative corn plants inoculated with *Bacillus megaterium* bacteria and un-inoculatd plants in the presence of rock phosphate with 0% level. **(B)** plants inoculated with *Bacillus megaterium* bacteria and un-inoculatd plants in the presence of rock phosphate with 50% level **(C)** plants inoculated with *Bacillus megaterium* bacteria and un-inoculatd plants in the presence of rock phosphate with 100% level .**(D)** Bacterial plates isolation for total count bacteria (CFU). All treatments without herbicide.

B) Effect of rock phosphate, herbicides and phosphate solubilizing bacteria on Chemical composition

It is evident from Table (4) that the different treatments showed a significant response to chemical characters. Data showed rock phosphate at 100% rate treatment recorded the highest chlorophyll index, maximum percentages of nitrogen, phosphorus and potassium (32.23 SPAD, 1.24, 0.60 and 1.48 %), as compared with control treatment which gave the lowest chlorophyll index, minimum percentages of nitrogen, phosphorus, and potassium (20.63 SPAD, 0.79, 0.38 and 0.94 %), consequently.

The results recorded on nitrogen, phosphorus and potassium percentages presented are in Table (4), revealed that nano-herbicides significantly increased chlorophyll index (31.89 SPAD), nitrogen, phosphorus and potassium percentages (1.22, 0.59 and 1.46 %), as compared with control treatment which recorded the lowest chlorophyll index value (20.92 SPAD), least percentages of nitrogen, phosphorus, and potassium (0.80, 0.39 and 0.96 %),It is clear from the obtained data in the same table that inoculation with phosphate solubilizing bacteria gave the highest chlorophyll index values, maximum percentages of nitrogen, phosphorus and potassium (27.59 SPAD, 0.95, 0.51 and 1.26 %), as compared with control treatment which recorded the lowest chlorophyll index, minimum nitrogen, phosphorus and potassium percentages (24.83 SPAD, 1.06, 0.46 and 1.13 %), respectively.

The interactions among rock phosphate and herbicides, rock phosphate and phosphate solubilizing bacteria were highly significant on nitrogen, phosphorus and potassium percentages. Also, the interaction between herbicides and phosphate solubilizing bacteria and the interaction between rock phosphate, herbicides and phosphate solubilizing bacteria were highly significant on nitrogen, phosphorus and potassium percentages and chlorophyll index. Kumar *et al.* (2001) suggested that the inoculation with bacteria capable to solubilize phosphates, phosphate rock which increases the availability of this element in the substrate, and as a consequence, increases its acquisition by the plant. The description above coincides with the results of the present study where it was shown that the use of phosphate rock as a source of P in maize crops with the inoculation of bacterial strains can increase up to 10 % the uptake of this element in comparison with the non-inoculated control supplied with phosphate rock. Phosphate solubilizing microorganisms solubilized P to be available-P with its ability to secrete organic acids that can break complex P compounds in the soil (Whitelaw, 2000).

The inoculation with microorganisms having phosphate solubilizing ability concurrently improved plant P uptake and crop growth. This increase in growth may be attributed to auxin production (Gyaneshwar *et al*., 2002; Fankem *et al.,* 2008), ACC-deaminase activity (Zafarul- Hye *et al*., 2007; Naik *et al*., 2008), production of organic acids (Fankem *et al*., 2008) or phosphatases (Abd-Alla, 1994; Chabot *et al*., 1996) to solubilize/mineralize P, thereby increasing phosphate nutrition of inoculated plants. Similarly, Kapri and Tewari (2010) got increased shoot /root length, fresh and dry weight of shoot/root of chickpea due to inoculation with phosphate solubilizing and phosphatase producing *Trichoderma* sp. Similarly, Linu *et al.* (2009) found that *Burkholderia* sp. gave better results in improving the growth of cowpea and this strain had been previously evaluated by Pandey *et al*. (2005) to have phosphate solubilization, auxin production, ACC deaminase activity and also nitrogen-fixing ability.

C) Total bacterial count cfu

The samples were spread on Pikovskaya (PVK) agar plates which contained insoluble phosphate. Although bacterial counts were similar between the rhizosphere of the corn (approximately 1 x 10^6 cfu ml^{-1}), the number of bacteria that created in the PVK aga, Figure (3) illustrated that the application of *Bacillus megaterium* increased the total bacterial count compared to the Un-inoculated plants, however the total count of bacteria decreased in the high levels of rock phosphate but recorded a greater amount in case of nanoherbicide. The recorded data was agreed with (Zafarul-Hye *et al*., 2007; Naik *et al*., 2008).

Table (4). Effect of rock phosphate, herbicides and phosphate solubilizing bacteria on chemical composition of corn plants

Treatments	Chlorophyll index (SPAD)	N (%)	P (%)	K (%)
A) Rock phosphate				
Control	20.63	0.79	0.38	0.94
50%	25.78	0.99	0.48	1.18
100%	32.23	1.24	0.60	1.48
LSD (0.05)	**0.42**	**0.01**	**0.001**	**0.001**
B) Herbicides				
Control (H1)	20.92	0.80	0.39	0.96
Herbicide (H2)	25.83	0.99	0.48	1.18
Nano-herbicide (H3)	31.89	1.22	0.59	1.46
LSD (0.05)	**0.20**	**0.004**	**0.0004**	**0.003**
B) PSB (*Bacillus megaterium*)				
Un-inoculated	24.83	1.06	0.46	1.13
Inoculated	27.59	0.95	0.51	1.26
LSD(0.05)	**0.04**	**0.001**	**0.0002**	**0.001**
Interaction				
A×B	**	**	**	**
A×C	**	**	**	**
B×C	**	**	**	**
A× B × C	**	**	**	**

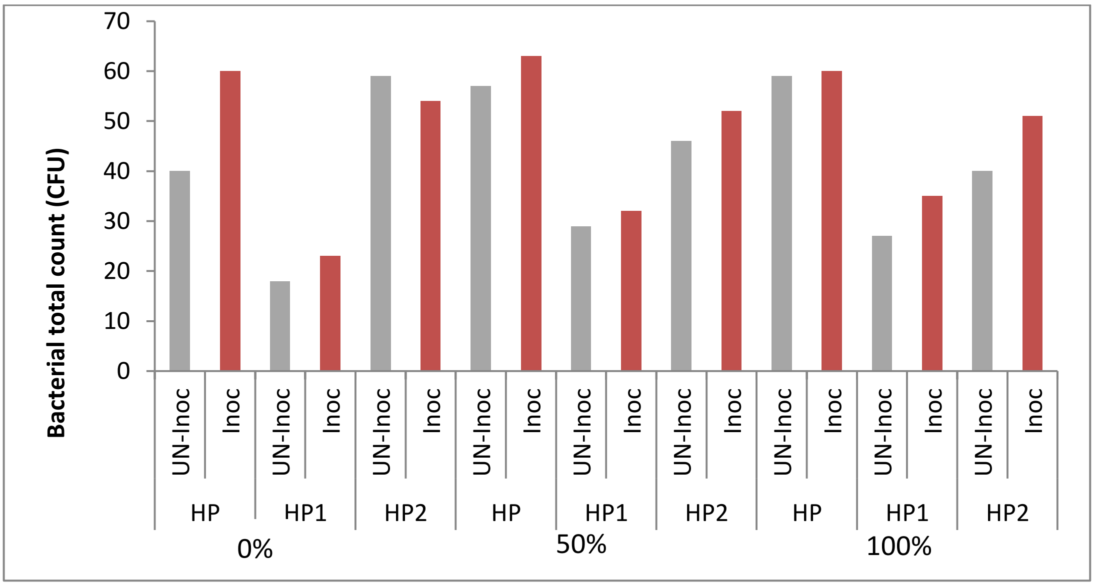

Figure (2b). Illustrate the total phosphate solubilizing bacteria and total count on PVK plate media, cfu taken with 10^{-6} concentration

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الملخص العربي

تأثير صخر الفوسفات وجسيمات مبيد الحشائش والبكتريا المذيبة للفوسفور علي النمو والمحتوي الكيماوي لنباتات الذرة المنزرعة في تربة جيرية

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أجريت تجربة أصص على الذرة في الصوبة التجريبية لكلية الزراعة (سابا باشا) ، جامعة الإسكندرية، مصر، لتقييم دور بعض مبيدات الحشائش ذات الجسيمات المتناهية الصغر على نمو الذرة. تم تصميم التجربة على أنها تصميم قطع منشقة مرتين مع أربع مكررات. كل مكررة تحتوي على ٨ معاملات على النحو التالي: صخر الفوسفات (كنترول، ٥٠%،١٠٠% م الجرعات الموصي بها) مبيدات الحشائش (كنترول، مبيدعادي، مبيد النانو) ، والبكتريا المذيبة للفوسفور{ (*Bacillus megaterium*) غير ملقح ، ملقح)}. أشارت النتائج إلى أن المعاملة بصخر الفوسفات بتركيز ١٠٠%، مبيد النانو والبكتيريا سجلت أعلى متوسط لقيم الصفات الخضرية مثل (إرتفاع النبات (سم)، الوزن الطازج والجاف لكلا من المجموع الخضري والجذري (جم)، الكلورفيل الكلي، دليل المساحة الورقية، وذلك مقارنة بالكنترول الذي أعطي أقل القيم لهذه الصفات، كذلك سجلت المعاملة بصخر الفوسفات بالمعدل ١٠٠%، مبيد النانو والبكتيريا أعلي القيم للوزن الطازج والجاف للجذر مقارنة بالنباتات غير المعاملة، من ناحية آخري، أدت المعاملة بصخر الفوسفات بالمعدل ١٠٠%، مبيد النانو والبكتيريا إلي الحصول علي أعلي النسب المئوية لكل من النيتروجين والفوسفور والبوتاسيوم في الأوراق، مقارنة بمعاملة الكنترول وبقية المعاملات الآخرى.