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Impact of application of rock phosphate (RP) inoculated with phosphate solubilizing fungi (PSF) as a fertilizer on total nitrogen (TN), total organic carbon (TOC) and microbial count (MC) in clay soil.

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ABSTRACT

In this study the effect of four treatments of phosphate fertilizers (NS, RP, SP & RP inoculated with PSF (*A. niger & A. fumigatus*) using three doses (RD, 1/2 RD&2RD) on TN, OC and MC of Nile delta clay soil and its impact on some growth parameters of *phasoleous vulgaris* was monitored. The Results indicated that OC content of clay soil was positively affected by the amendment of soil with inoculated RP. Inoculation of RP with *A.niger* culture increased the OC content of the soil by 4.91%, 18.28%, 14.38% and 19.61% comparing to *A.fumigatus*, NS, RP and SP respectively. the highest soil organic carbon content obtained when soil was amended with inoculated RP in 1/2RD it increased by 1.02% and 7.232% comparing to the amendment of with RD and 2RD. RP inoculated with A.niger culture clay soil showed the highest TN content when amended with 2RD the presence of *phasloeus vulgaris* plant it was 19.19%. Different phosphate treatments affected MC in clay soil to reach the highest count when inoculated rock phosphate with the RD as a fertilizer it increased by5.66% & 20.75% case of *A.fumigatus* & *A.niger* respectively comparing to NS. The lowest count obtained when SP was used as a fertilizer, it decreased by 26.4% comparing to NS.

Keywords: phosphate solubilizing fungi, phosphate biofertilizers, inoculated rock phosphate, soil organic carbon content, soil total nitrogen content.

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INTRODUCTION:

Phosphorus is the second important key plant nutrient after nitrogen. An adequate supply of phosphrous (P) is therefore required for proper functioning and various metabolisms of plants. RP enriched with compost and phosphate solubilizing microorganisms (PSMs) can be a good economical and ecofriendly way to increase the availability of (P) in soil. In most soil carbon (C)and nitrogen (N) is considered to be composed of several functional pools that differ both in their intrinsic degradability and in the factors controlling the relative decomposition rates (Stevenson, 1994); (Lützow et al., 2007). The addition of extra (P) in the soil led to consume more resources for the crop production, and hence, the costs of production of crop plants per unit area increase (Aziz, Rahmatullah, Maqsood, Tahir, & MA, 2006). Phosphorus deficiency affects not only plant growth and development and crop yield, but also the quality of the fruit and the formation of seeds (Njira & Nabwami, 2015) addition of P fertilizer increases grain yield in many crops such as tef (Deressa, Hassan, Alemu, Yesuf, & Ringler, 2008); (Bacha & Vinicios de Carvalho, 2014)wheat (Gunes, Inal, Alpaslan, & Cakmak, 2006)sorghum (Akram, Fatima, Ali, Jilani, & Asghar, 2007; Assem, Hussein, Hussein, & Basry, 2009), common bean (;Gidago et al., 2011; Ali et al., 2014; Kassa, *et al*., 2014) and soybean (Devi, *et al*., 2012), the excessive usage of P fertilizer has been associated with environmental pollution (Mengel, and Kirkby , 2001; Marschner, 2012). The work of Adnan, et al., (2017) indicated that Inoculation with phosphate-solubilizing bacteria (PSB) neutralizes the negative effects of soil calcification on the bioavailability of soil phosphorus (P) from mineral, natural and organic fertilizers. In the study held by Mickan, et al., (2018) it was proved that the amendment of soil with clay and compost to water stressed soil increased the resistance of rhizosphere bacterial community to water stress. In contrast, there were distinct responses in community composition to water stress within the unamended soil and for soil amendment with both clay and compost.

MATERIAL AND METHODS:

Preparation of inoculated rock phosphate:

Each of A. Fumigates and A. niger was grown at optimum solubilization conditions 28°C, 10 days incubation period, 1% ore concentration, initial pH 6.5-7, sucrose as a carbon source, NaNO3 as a nitrogen source for A. niger and NH4Cl for A. fumigates (Hefnawy et al.,2009). cultures were filtered, centrifuged at 3000rpm for 15 min, the fungal growth was washed, culture filtrate was mixed with washed fungal growth. The culture mixtures for A.niger and A. fumigate were kept separeatly in sterilized capped glass jars for further work. Finely grounded and sieved rock phosphate was mixed in a concentration of (30% w/v) with culture mixtures in penta replicate set for A. niger or A. fumigates in 500ml Erlenmeyer flasks, incubated in a rotary shaker at 120 rpm and 30ºC for 15 days incubation periods (Hefnawy et al.,2017).

Prepration of soil groups:

Clay soil was obtained from the delta region (Algharbia governorate) in Egypt ,air dried sieved and distributed in 5kg capacity plastic planting bags divided into 5 groups (inoculated rock phosphate (with *A. fumigatus* culture), inoculated rock phosphate (with *A. niger* culture), native soil (NS or No treatment), rock phosphate RP and super phosphate SP) each group has 3 subgroups of 1-recommended dose of fertilizer according to the Egyptian ministry of agriculture (RD) 2- half of the recommended dose (1/2RD) and 3- double of the recommended dose (2RD). Each treatment was prepared by means of penta replicates.

Determination of total nitrogen:

soil samples were subjected to persulphate digestion to determine total nitrogen method described by(US EPA method 600 /88. , (Greene, Bartels, Warren-Hicks, Parkhurst, & Linder, 1988) & (Prokopy, 1992).Stock acid

solution, 5.6M Sulfuric Acid: 310 mL of concentrated H2SO4 was diluted to 1 L with Double de-ionized water.

Working digestion acid solution: 12.8 g ammonium persulfate and 32 mLs of 5.6M H2SO4 were dissolved in a 100-mL volumetric flask. Dilute to mark with double de-ionized water.

.0250 g (~25 mg) approx of dried ground soil sample was Weighed. Sample was transferred to a caped tube Add 0.5 mLof working digestion acid to each tube. Vortex and covered with caps. Keep tubes loosely capped. Autoclave the digestion tubes for 1 hour (liquid cycle) at 121C and 15-20 psi. Remove tubes from autoclave, cool, and securely cap tubes. Allow any particulate matter to settle overnight. Then total nitrogen in the form of nitrate was determined colorimitrically .

Colorimetric assay of nitrate:

The amount of nitrate was determined colormetrically using brucine the method was described by (American Public Health, Taras, Water Pollution Control, & American Water Works, 1975) (Annual Book of ASTM Standards, "Water",Standard,1976). and (Jenkins & Medsker, 1964)).

Reagents: Distilled water free of nitrite and nitrate is to be used in preparation of all reagents and standards.

Sodium chloride solution (30%): Dissolve300g Na Cl in distilled water and dilute to 1liter.

Sulfuric acid solution: Carefully add 500mL conc.H2SO4 to125 ml distilled water. Cool and keep tightly stoppered to prevent absorption of atmospheric moisture.

Brucine-sulfanilic acidreagent: Dissolve 1gbrucine sulfate[(C23H26N2O4)2H2SO47H2O] and 0.1g sulfanilic acid(NH2C6H4SO3HH2O)in70mL hot distilled water.Add 3mL conc. HCl, cool ,mix and dilute to100mL with distilled water. Store in adark bottle at5°C. This solution is stable for several months; the pink color that develops slowly does not affect its usefulness.

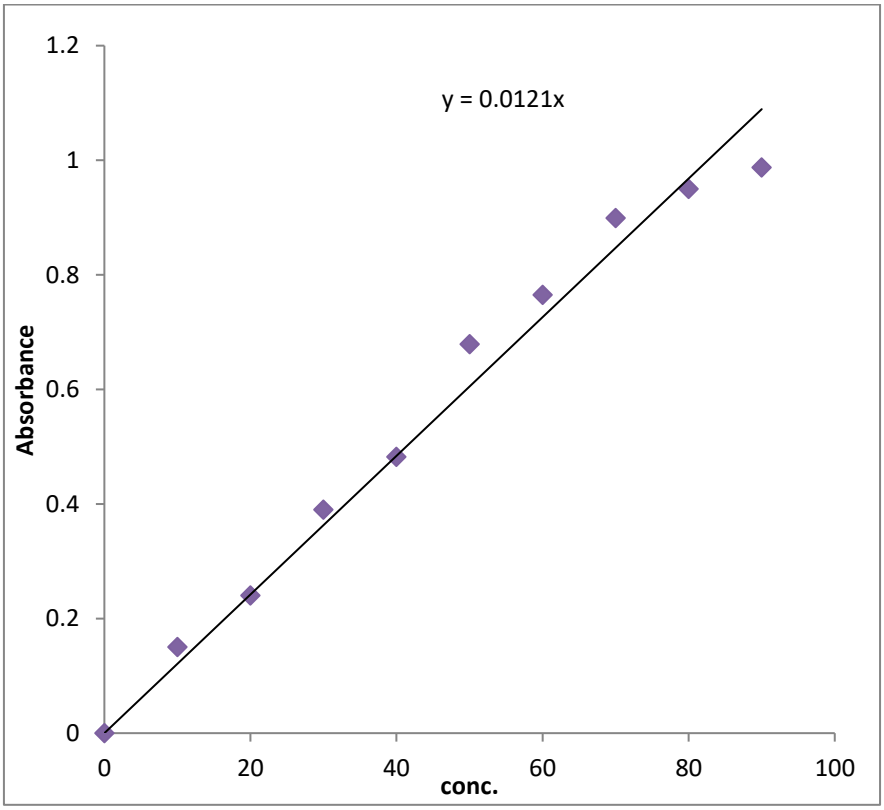

Fig 1 . Nitrate standard curve.

Determination of organic carbon:

Organic carbon was determined using the Titration method described by Black et al. (1965), (Bartlett, 1994), (McLeod, 1973). **Reagents: 1 N Potassium Dichromate**: 49.040 g K2Cr2O7 AR (dried at 105 °C)was dissolved in deionised water, transfered to a 1 L volumetric flask and make to volume with deionised water. **Sulphuric Acid 98% w/w:** This should be used fresh from the bottle and not left standing in a burette or beaker, as it rapidly picks up moisture from the air. It is satisfactory until the strength falls to <96%. **0.4 N Ferrous Sulphate:** 112 g FeSO4.7H2O was dissolved in 800 mL deionised water containing 15 mL concentrated H2SO4. Dilute to 1 L with deionised water and store in a dark bottle. **"Ferroin":** 1.485 g O-phenanthroline monohydrate and 0.695 g ferrous sulphate was dissolved in approximately 80 mL deionised water, then dilute to 100 mL. Store in a dark bottle away from light.

PROCEDURE: 1. Determine the moisture content of the air-dry soil which has been ground to pass a 0.42mm sieve.Weigh accurately enough soil to contain between 10 mg and 20 mg of carbon into a dry tared 250 mL conical flask (between 0.5 gm to 1 g for topsoil and 2 g and 4 g for subsoil). **2.** Accurately add 10 mL 1 N K2Cr2O7 and swirl the flask gently to disperse the soil in the solution. Add 20 mL concentrated H2SO4, directing the stream into the suspension. Immediately swirl the flask until the soil and the reagent are mixed. Insert a 200 °C thermometer and heat while swirling the flask and the contents on a hot plate or over a gas burner and gauze until the temperature reaches 135 °C (approximately ½ minute). **3.** Set aside to cool slowly on an asbestos sheet in a fume cupboard. Two blanks (without soil) must be run in the same way to standardise the FeSO4 solution. **4.** When cool (20–30 minutes), dilute to 200 mL with deionised water and proceed with the FeSO4 titration using either the "ferroin" indicator or potentiometrically.

"Ferroin" Titration": Add 3 or 4 drops of Ferroin indicator and titrate with 0.4 N FeSO4. As the end point is approached, the solution takes on a greenish colour and then changes to a dark green. At this point, add the ferrous sulphate drop-by-drop until the colour changes sharply from blue-green to reddish-grey. If the end point is overshot, add 0.5 or 1.0 mL of 1 N K2Cr2O7 and reapproach the end point drop-by-drop. Correct for the extra volume added. If over 8 mL of the 10 mL dichromate have been consumed, the determination must be repeated with a smaller soil sample.

CALCULATIONS: From the equation:

$2Cr_2O_7^{2-} + 3C + 16H^+ \rightarrow 4Cr^{3+} + 8H_2O + 3CO_2\uparrow$1 mL of 1 N Dichromate solution is equivalent to 3 mg of carbon.Where the quality and normality of the acid/dichromate mixture used are as stated in the method, the percentage carbon is determined from the following:

$$\text{Organic Carbon (\%)} = \frac{0.003 \times N \times 10 \times (1 - T/S) \times 100}{ODW}$$

$$= \frac{3(1 - T/S)}{W}$$

Where:-

N = Normality of $K_2Cr_2O_7$ solution

T = Volume of $FeSO_4$ used in sample titration (mL)

S = Volume of FeSO4 used in blank titration (mL)

ODW = Oven-dry sample weight (g).

Microbial count: Microbial count was determined by : Martin (1950)

. Ten, sterilized test tubes taken and the first test tube was taken 10 ml of distilled water, and remaining test tubes were with 9 ml distilled water. 1 g of sample was dissolved in a first test tube with distilled water (10 ml) and its dilution factor 10−1. Then, 1 ml of sample was transferred from first tubes (10−1) and second test tubes and its dilution factor is 10−2, similarly samples were transferred to remaining test tubes, and dilution factor obtained as 10−3,

10−4, 10−5, 10−6, 10−7, 10−8, 10−9, and 10−10.

Enumeration of microbial population of soil (bacteria and fungi) : About 1 g of soil is added into the distilled water taken in one of the test tubes. The content is mixed and allowed to sterile. If necessary, clear suspension is obtained by repeated soil extraction. Using a sterile, 1.0 ml pipette 2 drops of clear soil sample is spread on the nutrient agar and PDA surface of the plate. The sample is spread with a sterilized pour plate method. The culture plate is inoculated at room temperature for 24 hrs. for bacteria colony culture and 72 hrs. for fungal colony cultures. Then the formed colonies were counted using colony counter and the count was multiplied by inverted dilution.

RESULTS:

Effect of different phosphate treatments on clay soil organic carbon (TOC) content.

Organic carbon content of clay soil was positively affected by the amendment of soil with inoculated rock phosphate. Using of rock phosphate inoculated with A.niger increased the organic carbon content of the soil by 4.91%, 18.28%, 14.38% and 19.61% comparing to A.fumigatus, native soil (NS), rock phosphate (RP) and superphosphate (SP) respectively. Fig (2). Resultes revealed that highest soil organic carbon content obtained when soil was amended with inoculated rock phosphate in half of the recommended dose it increased by 1.02% and 7.232% comparing to the amendment of with recommended dose and double of the recommended dose . the lowest value of clay soil organic carbon content was shown when superphosphate was used as aphosphorus source. Data were subjected to analysis of variance (ANOVA TEST) Table (1).

Table(1): ANOVA test results for the effect of different phosphate treatment on clay soil total organic carbon content. Data are expressed as percentage.

Treatment		Minimum	Maximum	Sum	Mean Statistics	
		value	value	Statistic	Mean	Std. Error (±)
A. fumigatus	Inoculated RP(RD)	23.58	47.34	904.20	36.1680	1.70891
	Inoculated RP(1/2 RD)	20.22	45.60	832.68	39.6808	2.08843
	Inoculated RP(2 RD)	30.00	40.40	849.03	33.9612	.78720
A. niger	Inoculated RP(RD)	31.98	55.30	1089.34	43.5736	1.75525
	Inoculated RP(1/2 RD)	30.00	55.40	1039.81	44.5924	2.05985
	Inoculated RP(2 RD)	30.00	45.55	933.95	37.3580	1.23089
Controll	Native soil	18.00	33.42	657.66	26.3064	1.28735
	Rock phosphate (RP)	23.00	38.00	755.31	30.2124	1.11011
	superphosphate(SP)	16.90	35.55	624.51	24.9804	1.45107

*RD Recommended dose of the fertilizer according to the Egyptian ministry of agriculture.

*Native soil (NS) Is soil without any treatments.

*± stand error of mean of penta replicates.

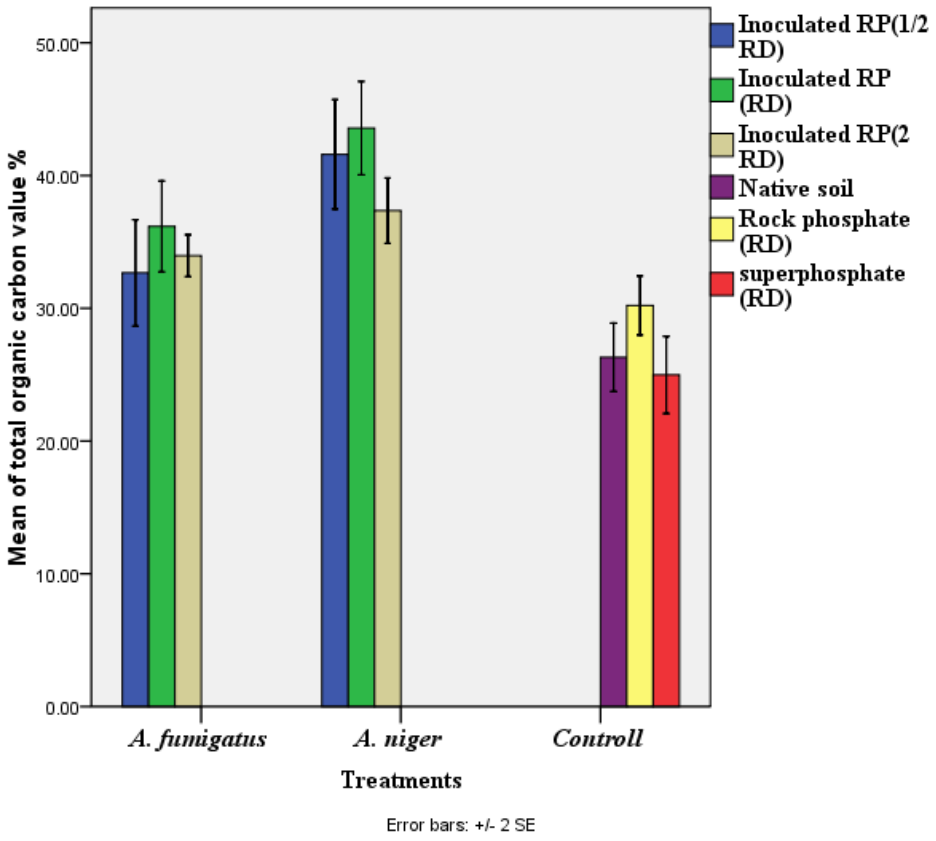

Fig.(2): Effect of different phosphate treatments on clay soil total organic carbon content

*RD Recommended dose of the fertilizer according to the Egyptian ministry of agriculture.

*Native soil (NS) Is soil without any treatments.

*± stand error of mean of penta replicates.

Effect of different phosphate treatments on clay soil total nitrogen (TN)content.

Total nitrogen content of clay soil was significantly enhanced by the amendment of soil with inoculated rock phosphate. It was increased in case of using rock phosphate inoculated with *A.fumigatus* culture greater than that inoculated with *A. niger* culture. In the case of inoculated RP with *A.niger* culture clay soil showed the highest total nitrogen content was 19.1908%when amended with double of the recommended dose fig(3).Data were subjected to analysis of variance (ANOVA TEST) Table (2).

Effect of different phosphate treatments on microbial count (MC) as well as phosphate solublizers count of clay soil.

Results indicated that different phosphate treatments considerably affected microbial count and diversity in clay soil to reach the highest count in the case of using inoculated rock phosphate with the recommended dose as a fertilizer it increased by5.66% & 20.75% case of *A.fumigatus fig.(4) and A.niger* fig. (5) respectively comparing to native soil fig (6).The lowest count obtained when super phosphate was used as a fertilizer fig. (7) , the microbial count decreased by 26.4% comparing to native soil, while the use of rock phosphate as a fertilizer slightly affected the microbial count of clay soil fig.(8). On the other hand the percentage of phosphate solubilizing organisms reached the highest value in case of rock phosphate as a fertilizer it increased by 12% comparing to native soil and by 0.3% &3% comparing to *A.fumigatus and A.niger* respectively, while it exceeded the percentage by 9% in case of super phosphate

Table (2): ANOVA test results for the effect of different phosphate treatment on clay soil total Nitrogencontent. Data are expressed as percentage.

Treatment		Minimum	Maximum	Sum	Mean Statistic	
		value	value	Statistic	Mean	Std. Error (±)
A .fumigatus	Inoculated RP(RD)	15.11	19.67	434.55	17.3820	.23857
	Inoculated RP(1/2 RD)	9.33	15.77	291.06	11.6424	.33851
	Inoculated RP(2 RD)	9.50	12.11	269.05	10.7620	.16128
A. niger	Inoculated RP(RD)	9.01	26.54	408.16	16.3264	1.53086
	Inoculated RP(1/2 RD)	12.98	16.77	348.99	13.9596	.23608
	Inoculated RP(2 RD)	12.77	29.77	479.77	19.1908	1.37955
Controll	Native soil	8.99	10.80	234.13	9.7554	.11392
	Rock phosphate (RP)	13.22	17.23	358.82	14.9508	.27294
	Super phosphate(SP)	11.89	19.77	362.16	15.0900	.65577

*RD Recommended dose of the fertilizer according to the Egyptian ministry of agriculture.

*Native soil (NS) Is soil without any treatments.

*± stand error of mean of penta replicates.

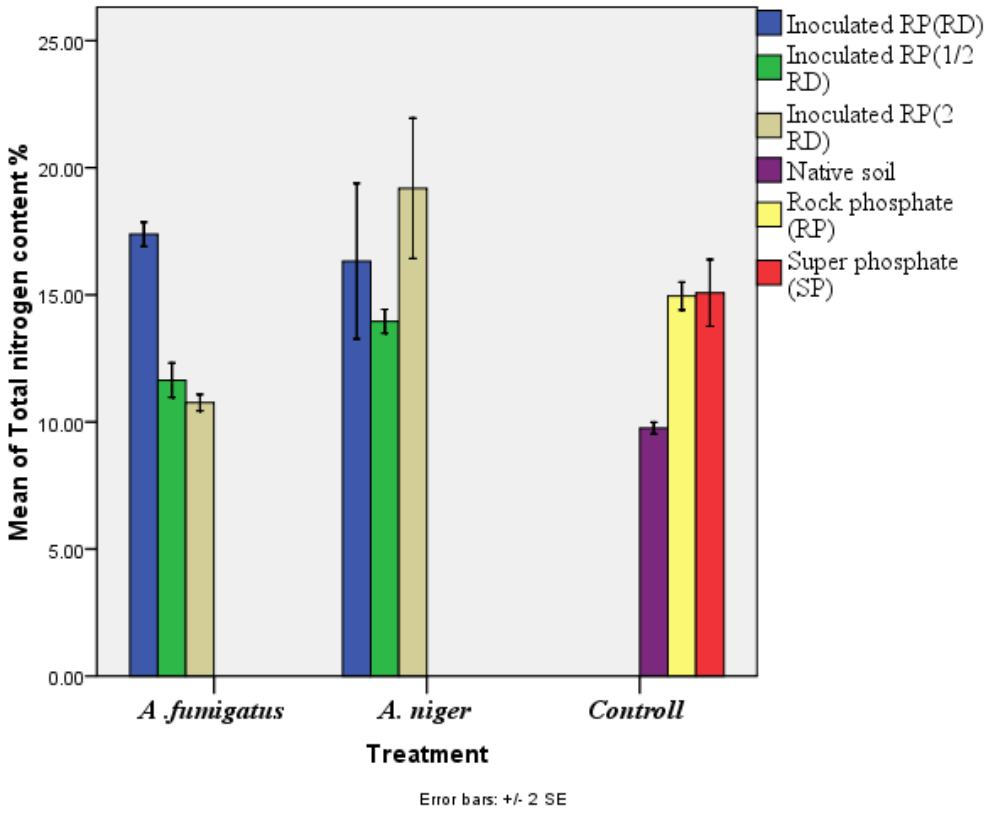

Fig.(3): Effect of different phosphate treatments on clay soil total Nitrogen content .

*RD Recommended dose of the fertilizer according to the Egyptian ministry of agriculture.

*Native soil (NS) Is soil without any treatments.

*± stand error of mean of penta replicates.

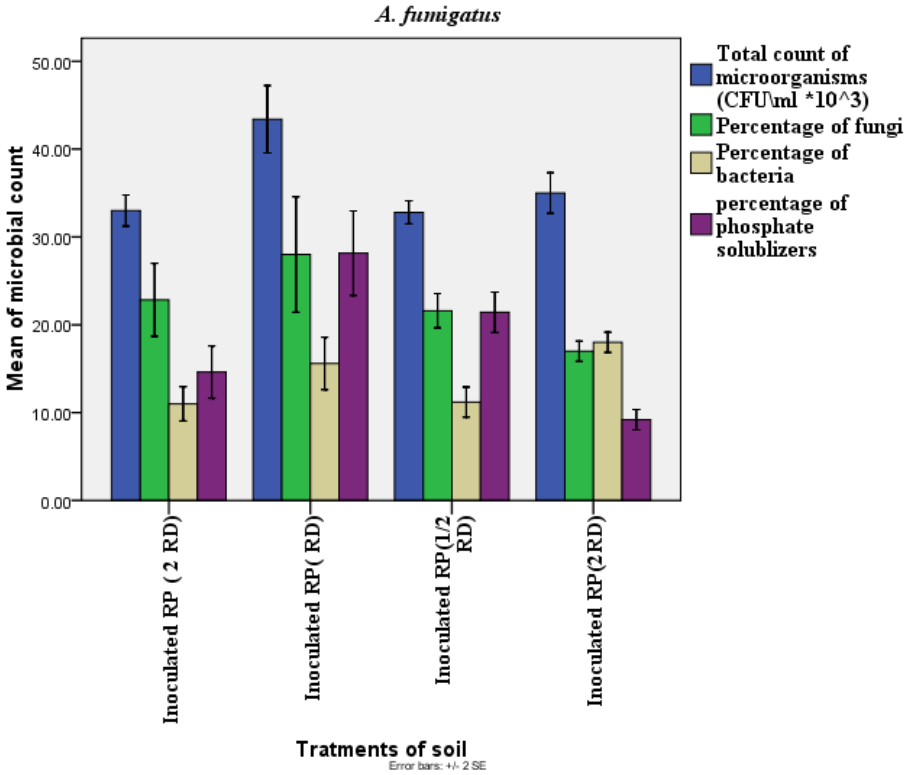

Fig.(4) Effect of different doses of inoculated rock phosphate with *A.fumigatus* culture on microbial count of clay soil .

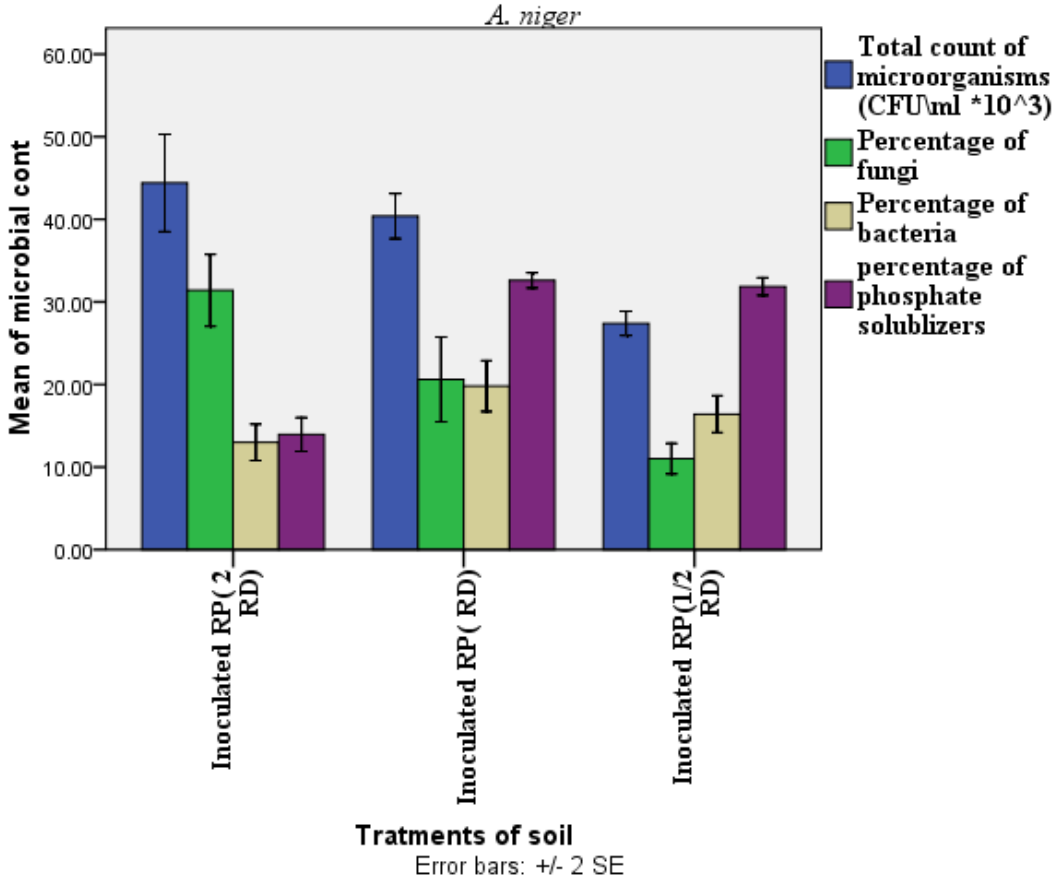

Fig.(5) Effect of different doses of inoculated rock phosphate with *A.niger* culture on microbial count of clay soil .

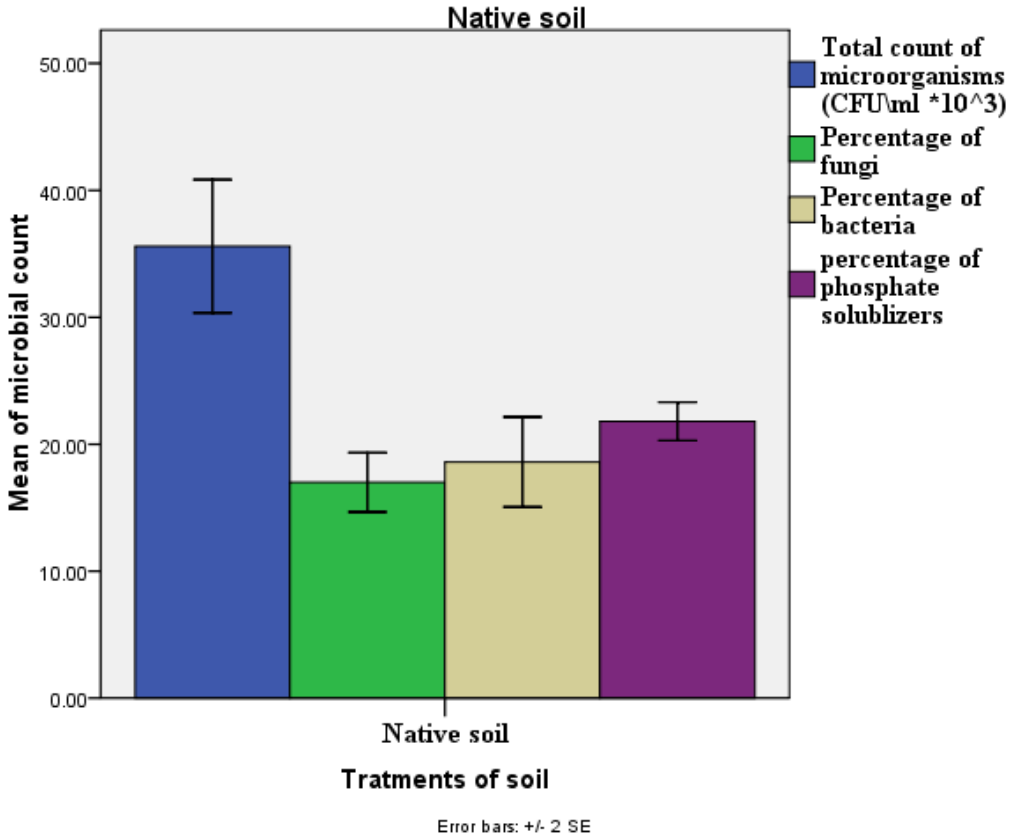

Fig.(6) Effect of Native soil (NS) on microbial count of clay soil.

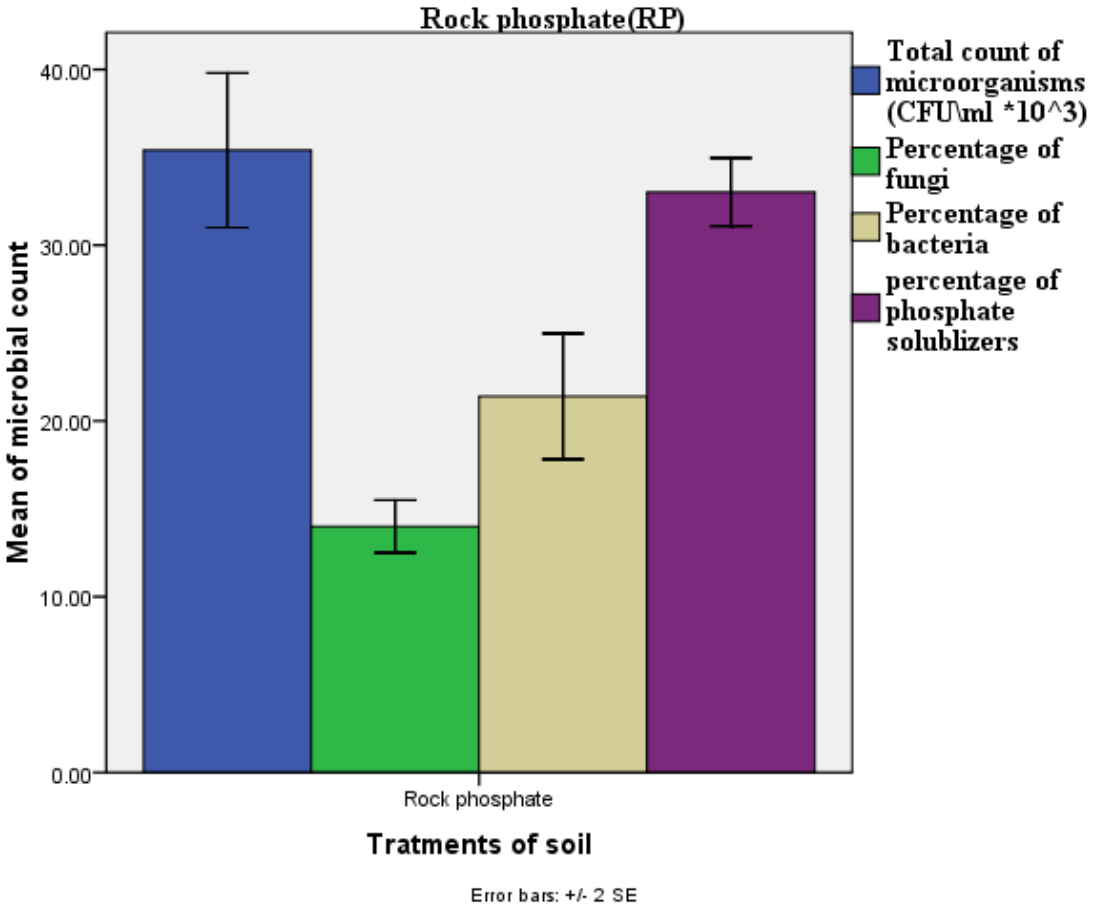

Fig.(7) Effect of rock phosphate (RP) on microbial count of clay soil.

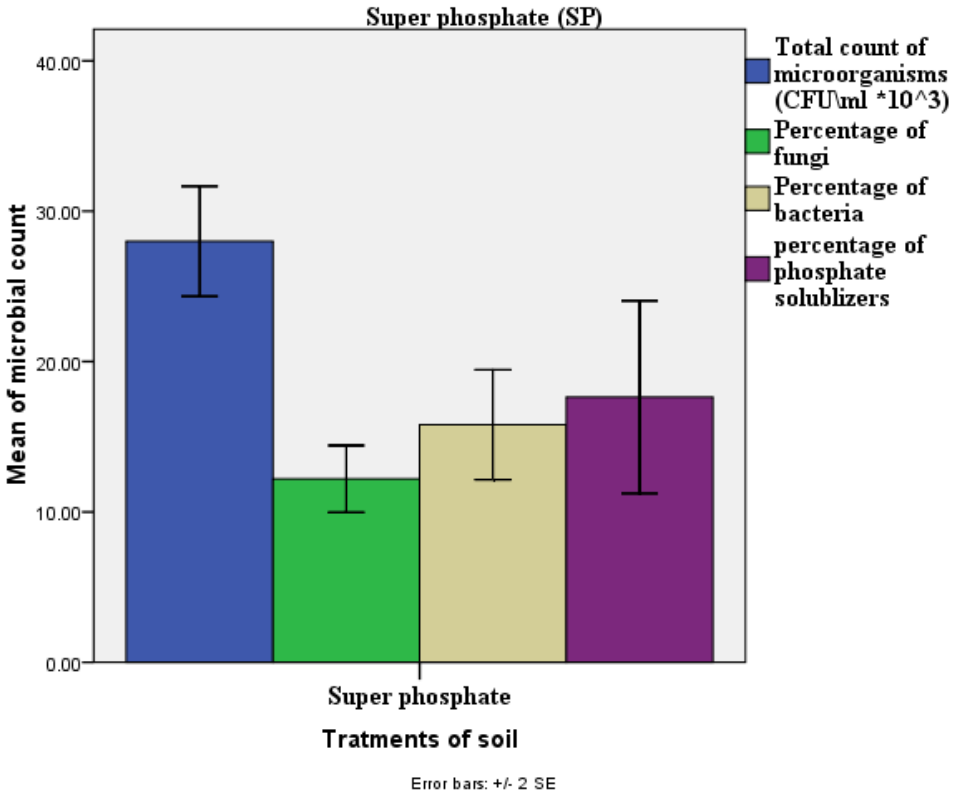

Fig.(8) Effect of super phosphate(SP) on microbial count of clay soil.

DISCCUION:

Phosphate solubilizing micro-organisms (PSMs) are abundant in soils they could play an important role in enhancing soil P availability in a more environmentally friendly and sustainable manner. Although solubilization of P compounds by microbes is very common under laboratory conditions, results in the field have been highly variable. This variability has encountered the large-scale use of PSMs in agriculture. This variability suggested to be caused by many reasons, but none of them have been extensively investigated. In the present study the effect of using RP inoculated with phosphate solubilizing fungi A.niger and A.fumigatus separately as a bio fertilizer on organic Carbone and total nitrogen levels as well as the count of microbial community in clay soil and the impact of this on some growth parameters of phasoloeus vulgaris plant. Obtained results indicated that utilization of inoculated rock phosphate considerably enhanced both s organic carbon and total nitrogen content these results agreed with the results obtained by Moharana, Meena, and Biswas (2018)whose results indicated that the use of Phosphate-Solubilizing Microbes enhanced phosphate availability as well as decreasing carbon-to-nitrogen (C/N) ratio of crop residues. Also it was proved that Applications of compost and clay to ameliorate soil constraints such as water stress positively affected the rhizosphere bacterial community and its functional component involved in nitrogen (N) cycling and soil carbon (C) degradation. Compost soil treatments increased the relative abundance of copiotrophic bacteria, decreased labile C and increased the abundance of recalcitrant C degrading genes(Mickan et al., 2018). On the other hand , results from this study proved that using of inoculated RP as a source of phosphorus had appositive impact on growth parameters of phasoloeus vulgaris plant such as shoot height ., no of flowers , no of fruits and leaves area ,the same results obtained by Ditta et al. (2018) ,Raliya, Tarafdar, and Biswas (2016),Shabnam and Iqbal (2016) , Wahid et al.

(2016) ,Elias, Muleta, and Woyessa (2016) and Youssef, Riad, and Abd Elhady (2017)the all proved that the amendment of soil with inoculated RP or phosphate solubilizing microorganisms, organic manure or compost considerably enhanced phosphorus availability in soil as well as other nutrients content of soil such as organic Carbone and total nitrogen it also enhanced plant up take of nutrients which have positive impact on the growth of many crop plants e.g. check beans, mug beans, wheat plant, maize and phasoloeus vulgaris plant as well as enhancing the diversity of soil microbial community.

Conclusion:

Results obtained in this study revealed that the use of rock phosphate inoculated with (PSF) cultures had a considerable positive effect on nutrient availability in soil as well as nutrient uptake by the plant that will have a positive impact on plant growth parameters.

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