Plant Archives Volume 20 No. 2, 2020 pp. 8863-8876 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

THE EFFECT OF USING NANOPARTICLES PHOSPHORUS AND ZINC ON QUALITY AND QUANTITY OF SOYBEAN (*GLYCINE MAX* L.)

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Abstract

In order to investigate the possibility of using Nano-technology to reduce the amounts of chemical fertilizers, field experiments were conducted in two locations in Sids Experimental Farm, ARC and El Fashn City, Beni-Suef Government. The design of the experiment was split – split design in three factors as follow A: chemical phosphorus fertilizer, *i.e.*, 0.0, 25, 50, 75 and 100 % from its recommended rate (RRP). B: foliar spraying of natural rock P Nanoparticles (with and without). C: zinc application (0.0 foliar spraying of 0.2% twice from zinc sulphate and foliar spraying of 0.2 % zinc oxide Nanoparticles twice). The results showed that increasing chemical phosphorus fertilizer up to 100% RR increased seed and straw yields, protein percentage and yield as well as oil percentage and yield. Foliar spraying of natural rock P Nanoparticles exhibited the highest soybean seed yield, protein percentage and yield, oil percentage and yield over control. Foliar spraying of zinc oxide Nanoparticles recorded the maximum seed and straw yields, protein percentage and yield and oil percentage and yield which equal to those under foliar spraying of zinc sulphate as compared with no zinc. The interaction between treatments revealed that, the treatment of 50% RRP+ foliar spraying of neutral rock P Nanoparticles+ foliar spraying of $ZnSO_4$ or ZnO nanoparticles gave the highest seed and straw yields, protein (%) and protein yield as well oil (%) and oil yield, which statistically equal to those under 100% RRP plus foliar spraying of zinc. Also, Nano-zinc oxide produced the studied traits equal to refer to zinc sulphate. These results mean that it could be saved about 50% RRP by using rock P Nanoparticles, beside the possibility of using zinc oxide Nanoparticles instead of zinc sulphate.

Key words: *Chemical phosphorus fertilizer, Nano-natural rock P, Nano-zinc oxide, soybean.*

Introduction

Generally, soybean (*Glycine max* L.) is the most important crop in the world due to its total production and international trade. It represents about 30% of the world processed crop oil and consider as bio-diesel fuels. It is recognized as “Golden Bean” due to its high nutritional value and its differentiation of use. The seeds of soybean contain about 20% oil and 40% good protein quality. Also, soybean seeds contain certain essential amino acids, soluble vitamins, priced product as lecithin about 20.5% starch, calcium, iron and vitamin B. Beside its nutritional value, soybean as legume plant used as N fixing crop, which can have fixed from 0–185 kg Nh^{-1} with an average with 84 kg Nh^{-1} (Russell and Birg 2004). It is used in soy sauce, soy yoghurt, soy milk, tofu (soybean curd), flour and some beverages. Soybean is a source of oil and protein, which is cheaper than animal protein.

Agricultural is most important to meet the increasing in worlds population, where it grows to about 8 billion people by 2025 and 9 billion by 2050 and it is widely nutrition security is to feed this population with food (Quasem *et al.*, 2009 and Ghaly 2009). Many researchers work to develop an efficient and ecofriendly production technology based on innovative technologies. In this concern, nanotechnologies can play an important role in increasing quality and quantity of foods. Nano-materials are defined as materials with single unit ranged between 1 to 100 Nano-meter in size in one dimension at least (Liu and Lal, 2015). Nano fertilizers are Nano materials added to plants to supply it with one nutrient or more to improve its growth and yield, also it facility for better

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performance of conventional fertilizers. Many workers proved the beneficial effect of Nano fertilizers, they reported that these materials have high nutrient use efficiency which improved yield and reduced soil pollution such as (Naderi and Danesh – Sharaki 2013 and Khan *et al.*, 2018).

Phosphorus is an essential macronutrient for plants. It is involved many processes for plant growth, such as cell division, development of good root system, it enhanced pod setting and seed formation, protein synthesis and improved N-fixation by root modules as a result of activate the symbiotic bacteria. Phosphorus also plays a vital role in virtually every plant process that related to energy transfer. It is important nutrient for biosynthesis of chlorophyll and essential for cell division and form the meristematic tissue. Accordingly, phosphorus deficiencies resulted in a reduction in the rate of leaf expansion and photosynthesis rate, hence reduction in yield (Kumar *et al.*, 2016). Phosphorus fertilizer such as mono ammonium phosphate, di ammonium phosphate or triple superphosphate are water soluble form for plant uptake. Also, these forms are very mobile in the soil and large portion of it ends up in surface water resulting in losing. However, solid phosphorus forms such as natural rocks have also been attempted to P fertilizers. These solid forms have a large size of particles, consequently less available for plant uptake. For this concern, Nano-size rock phosphate could be more effective in supplied P for plant beside minimizing the secondary contamination risk (Liu and Lal, 2015).

Zinc is essential micronutrients for plant growth and reproduction. It has several functions in plant such as enzyme activation and regulation, protein formation, photosynthesis, carbohydrate assimilation, fertility and production of seeds (Marschner, 1995). Zinc deficiency affected these physiological processes and comprise the health and productivity of plant. Zinc sulphate and chelate were used as zinc fertilizers added to plants whether as soil or foliar application, however, its efficiency is low. Moreover, zinc sulphate fertilizer has highly cost comparing with zinc oxide as a source of Zn. Therefore, more studies are needed on using Nano-scale ZnO particles for improve its efficiency for better uptake and crop production. For increase ZnO availability, it needs to smaller size, higher specific surface area and Nano-particles of size oxide.

Many workers stated the possibility of using Nano-fertilizers as total or partial substitution from chemical fertilizers, such as (Liu and Lal 2014 and Soliman *et al.*, 2016) for Nano-phosphorus and (Olkhovych *et al.*, 2016, Singh *et al.*, 2017 and Venkata *et al.*, 2018) for Nano-zinc.

Due to the unavailability of P and Zn in the alkaline soils of Egypt and because of its importance as two essential nutrients for plant growth, therefore the aim of this work is to evaluate the effect of application of different levels and sources of P and Zn whether conventional or Nano-materials on quality quantity of soybean plant.

Materials and Methods

The experiment was carried out during summer of 2018 (1 April) in the agriculture research Centre, Horticulture institute, Giza, to study the effect of application of yeast extract, on growth, yield and fruit quality of tomato (*Lycopersicon esculentum* L.) plants grown in a soilless agriculture system. Dry yeast was obtained from local market) Fares Alhaj *et al.*, 2017).

Plant material and soil analysis

Field experiments were performed during 2018 cropping season at two locations, the first location at Sids Agricultural Research Station, ARC, Beba City and the second location at a private farm in El Fashn City, both sites located in Beni-Suef Governorate, Egypt to assess the possibility of totally or partially substitute of chemical P and Zn by using Nano-natural rocks of P and Zn and its effect of quality and quantity of soybean plant (*Glycine max* L.). The soil of the two locations is clay loam in texture, had 7.8 and 8.0 pH, 1.01 and 1.13 EC (dSm^{-1}) and 1.65 and1.68 % organic matter, as well as 22 and 20; 11and 10; 185 and 173; 0.7 and 0.5 ugg^{-1} soil available N, P, K and Zn, respectively (according to A.O.A.C, 1990).

Field experimental design

The experiments were laid out in split – split plot design in three factors in complete randomized block with four replications. The factors were: (A) chemical phosphorus fertilizer (0.0, 25, 50, 75 and 100 % from recommended rate, 24 kg P ha^{-1}) were located in main plots. (B) Nano rock phosphate (without and 2 % foliar spraying of Nano rock phosphate twice after the second irrigation and after one month later we were located in the subplots). and (C) zinc treatment (without, foliar spraying of 0.2 % zinc sulphate and Nano-zinc oxide twice after the second irrigation and after one month later; we were applied in sub – subplot. Chemical phosphorus treatment was added during land preparation as superphosphate (15.5 P2O5%). The used Nano- rock P and Nano-zinc oxide had average particle size less than 30 nm and specific surface of particles more than 30 m^2g^{-1}. Nano rock P and Nano zinc oxide were prepared by Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef Univ, Egypt. The seeds of soybean for all plots were inoculated with specific Rhizobium Japonica strain by using Arabic gum and 1 % glucose (w/w) for activating the product candidates (were proved by Department of Microbiology, Soil, Water and Environment Institute, ARC, Egypt).

Seeds of soybean, variety Giza 111 were sown at 10 and 15 June in the two locations, respectively in hills 5 cm a part on ridge, 60 cm width, the plot size was 10.5 m2 (3×3.5 m). After 21 days from sowing the plants were thinned to one plant per hill. The recommended cultural practices for soybean in district were done. At maturity all plot area for all plots were harvested to determine seed yield/plot and converted to kg ha^{-1}.

Protein and oil determination

Nitrogen and oil percentages in seeds were determined according to (A.O.A.C, 1990) and converted to percent and totally yield of protein and oil.

Statistical analysis

All data were subjected to the statistical analysis according the methods of (Snedecor and Cochran 1980). The means of treatments were compared by L.S.D at 0.05 test.

Preparation and characterization of fertilizers nanoparticles (Rock phosphate and zinc oxide)

The nanoparticles of rock phosphate and zinc oxide were prepared in Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Egypt. The required nanoparticles were prepared separately using ball milling for 10 hours under the conditions mentioned in table 1 (Farghali *et al.,* 2007, Mahmoud *et al.,* 2017 and Shahin *et al.,* 2015).

A Panalytical (Empyrean) X-ray diffractometer was used to record the X-ray diffraction (XRD) patterns of rock phosphate and zinc oxide, in the angle range of 5-80°, with step scan of 0.02°, using Cu Kα1 radiation (λ = 1.5406 Å) at an acceleration voltage of 40 kV, with current of 30 mA,

The size and shape of nanoparticles was observed using high resolution transmission electron microscope (HRTEM JEOL-JEM 2100, Japan) using an accelerating voltage of 200 kV.

Table 1: Ball milling conditions for preparing nanoparticles.

Condition	Description
Balls diameters	ranged from 1.11 to 1.75 cm diameter
Vessel size	7.5cm diameter
Materials of balls	Porcelain
Materials of vessels	Stainless steel
Speed Ball	300 rpm
Precipitate mass ratio	8.1 mass ratio
Time	10h

Results and Discussion

Characterization of rock phosphate nanoparticles

XRD pattern of rock phosphate nanoparticles prepared by ball milling Fig. 1 revealed excellent crystallinity as strong reflections and large number of peaks are observed. By comparing the obtained pattern and matching with ICDD number 00-009-0363, the pattern was indexed and the nanoparticle adopted anorthic structure. The rock phosphate is one of the well-known calcium phosphate of the general formula Ca $(PO_3)_2$. The crystallite size was calculated from the Debge Scherer's equation.

$$L = 0.9\ \lambda / \beta\ cos\ \theta$$

When λ is the target wave length of the x-ray, β is

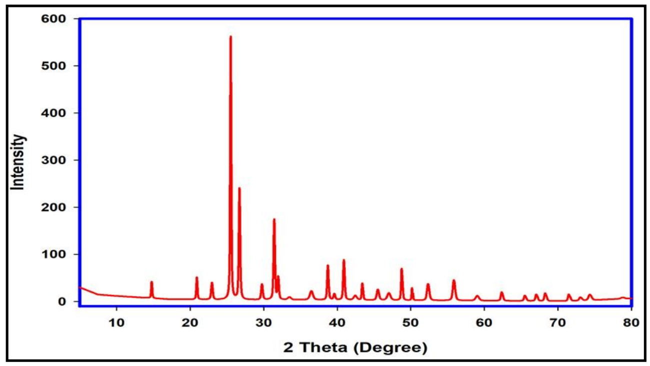

Fig. 1: XRD pattern of ca$(po_3)_2$ Nanoparticles (Rock phosphate).

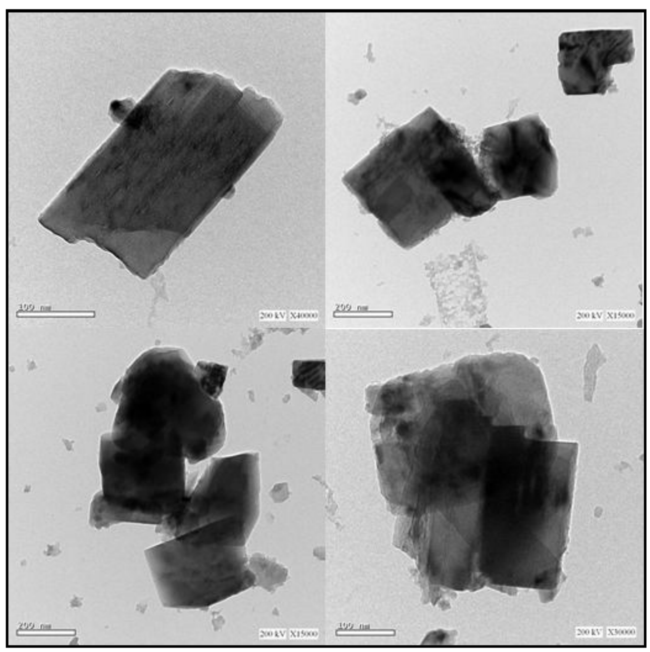

Fig. 2: TEM images of Ca$(PO_3)_2$ Nanoparticles (rock phosphate) at different magnifications.

the corrected full width at half maximum and θ is the diffraction angle. The rock phosphate prepared nanoparticles are in the 64 nm average range.

High resolution transmission electron microscope micrographs of rock phosphate nanoparticles are represented in Fig. 2. It is clearly seen that the rock phosphate is formed in a network of sheets stacked together. The sheets are of dimensions of few hundreds of nanometers but with very small thickness. No preferred orientation of the nanosheets was remarked.

Characterization of zinc oxide nanoparticles

XRD pattern of zinc oxide nanoparticles prepared by ball milling Fig. 3 revealed excellent crystallinity as strong reflections and large number of peaks are observed. By comparing the obtained pattern and matching with ICDD number 04-015-4060, the pattern was indexed and the nanoparticle adopted hexagonal structure with space group P36mc. The zinc oxide is one of the well-known oxides of the general formula ZnO. The average crystallite size was calculated and found to be 65 nm.

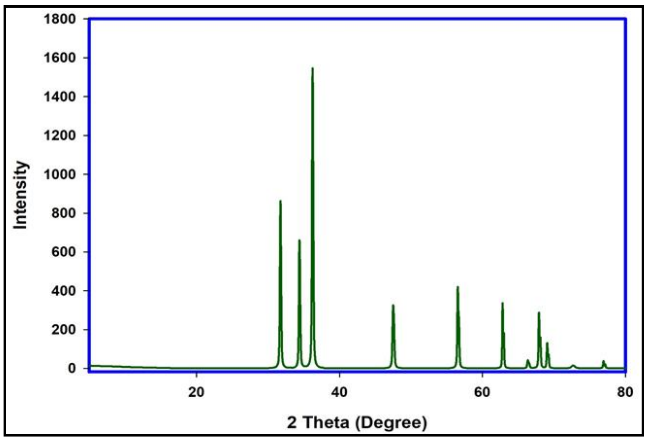

Fig. 3: XRD pattern of ZnO Nanoparticles (Zinc oxide).

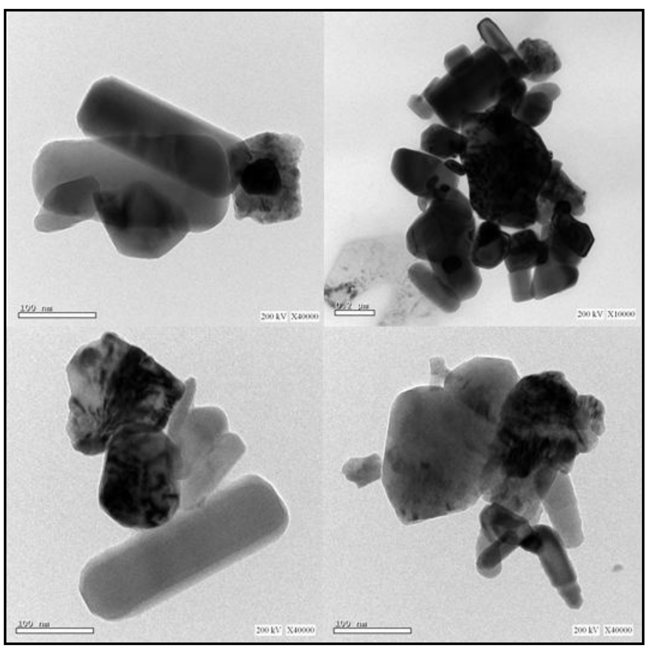

Fig. 4: TEM images of ZnO Nano particles (zinc oxide).

High resolution transmission electron microscope micrographs of zinc oxide nanoparticles are represented in Fig. 4. It is clearly seen that the zinc oxide is formed in a network of sheets stacked together. The sheets are of dimensions of few hundreds of nanometers but with very small thickness. No preferred orientation of the Nano sheets was remarked. The observed microstructure agrees well with the XRD analysis of the powder.

Effect of different fertilization practices on soybean yield

The effects of chemical and Nano-fertilizers of P and Zn and their interactions on seed and straw yields are given in tables 2 and 3 and illustrated in Figs. 5, 6, 7, 8, 9, 10, 11 and 12. As for the main effect of chemical fertilizers, the data clearly reveal that soybean grain and straw yields were significantly increased as chemical P fertilizer increased up to 100% recommended rate (RR) in both locations. The relative increasing of seed yield due to 100% RR of P reached to 97.3 and 112.75 % over without phosphorus application in the two locations, respectively. Similar trends were obtained for straw yield. The positive effect of phosphorus on increasing soybean yield may be due to the direct effect of P on improving N2 - fixation, model formation and nitrogen as activity, root formation, photosynthesis, flowering, seed formation (Ogoke *et al.*, 2003). These results are in line with those obtained by (Ismail *et al.*, 2014 and Suman *et al.*, 2018).

Concerning the main effect of Nano-natural rock P (Nano-RP), the data indicate that both seed and straw yields were positively responded to Nano- RP. Seed yield was increased by about 26.1 and 33.8 % as compared with no Nano- RP application in both locations, respectively. The increment in straw yield take the same trend. These increments may be attributed to the beneficial effects of Nano-particles which resulted to its high activity because high specific surface area, more density of reactive area, consequently easily absorbed by plants (Siddiqui *et al.,* 2015). These results are in similar to those obtained by (Liu and Lal 2014 and Soliman *et al.*, 2016).

Respecting the main effect of zinc treatment, the data indicate that, foliar spraying of zinc, whether zinc sulphate of Nano-zinc oxide were significantly improved seed and straw yields. It is worthy to notice that the difference between the effect of zinc sulphate and zinc oxide Nanoparticles was not significant. Foliar spraying of Zn SO_4 or zinc Nanoparticles increased seed yield by

Table 2: Effect of chemical and Nano-fertilization of phosphorus and zinc on seed yield (kg ha^{-1}) of soybean plant.

P		Zn (C)							
Chemical P	Nano rock P	1st Location				2nd Location			
		0.0	Sulphate	Nano	mean	0.0	Sulphate	Nano	Mean
0.0	0.0	1275.30	1450.70	1448.30	1391.43	1187.10	1344.40	1341.20	1290.90
	Nano	1969.10	2146.90	2147.10	2087.70	1813.20	1983.40	1986.40	1927.66
Mean		1622.20	1798.80	1797.70	1739.56	1500.15	1663.90	1663.80	1609.28
25%	0.0	1413.40	1591.40	1589.00	1531.26	1335.50	1494.30	1491.50	1440.43
	Nano	2542.60	2695.30	2697.10	2645.00	2379.60	2548.60	2550.10	2492.76
Mean		1978.00	2143.35	2134.05	2088.13	1857.55	2021.45	2020.80	1966.60
50 %	0.0	2058.30	2263.70	2262.30	2194.76	1957.60	2117.70	2119.20	2064.83
	Nano	3366.10	3462.10	3463.10	3430.43	3238.00	3512.30	3516.70	3422.33
Mean		2712.20	2862.90	2862.70	2812.60	2597.80	2815.00	2817.95	2743.58
75 %	0.0	2748.30	2914.00	2916.40	2859.56	2607.20	2825.40	2827.20	2753.26
	Nano	3369.40	3456.40	3465.50	3430.43	3235.20	3514.60	3517.60	3422.46
Mean		3058.85	3185.20	3190.95	3145.00	2921.20	3170.00	3172.40	3087.87
100 %	0.0	3365.50	3456.30	3465.40	3429.06	3239.90	3516.80	3516.90	3424.53
	Nano	3368.10	3466.20	3467.40	3433.90	3236.10	3515.20	3517.80	3423.03
Mean		3366.80	3461.25	3466.40	3431.48	3238.00	3516.00	3517.35	3423.78
mean of nano P	0.0	2172.17	2337.02	2336.28	2281.82	2065.46	2259.72	2259.20	2194.79
	Nano	2923.06	2660.78	3048.04	2877.29	2780.42	3014.82	3017.72	2937.56
mean of Zn		2547.61	2690.30	2692.16	2643.35	2422.94	2637.27	2638.46	2566.22
LSD at 5 %	A				36.5				39.8
	B				34.1				36.4
	C				23.6				29.1
	AB				40.3				40.1
	AC				NS				NS
	BC				NS				NS
	ABC				NS				NS

Effect of chemical and Nano-fertilization of phosphorus and zinc on seed yield (kg ha^{-1}) of soybean plant.

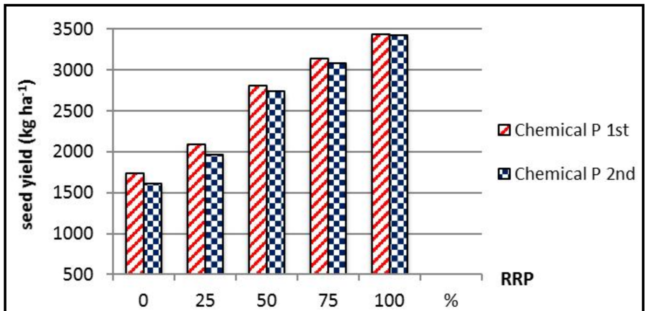

Fig.(5) : The main effect of chemical of phosphorus on seed yield

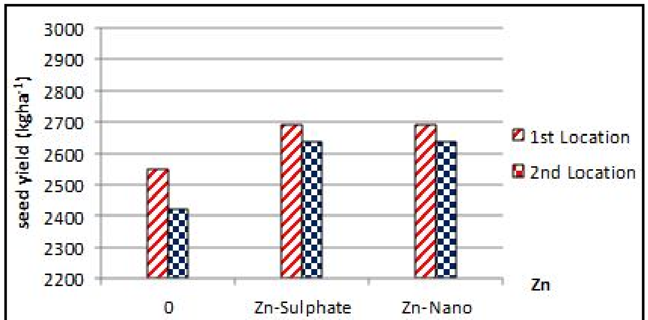

Fig.(7): The main effect of zinc on seed yield

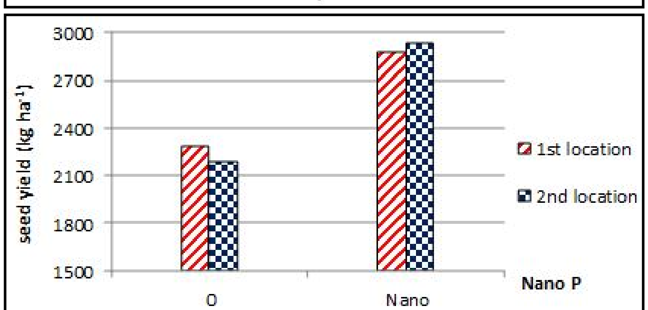

Fig.(6): The main effect of nano-fertilization of phosphorus on seed yield

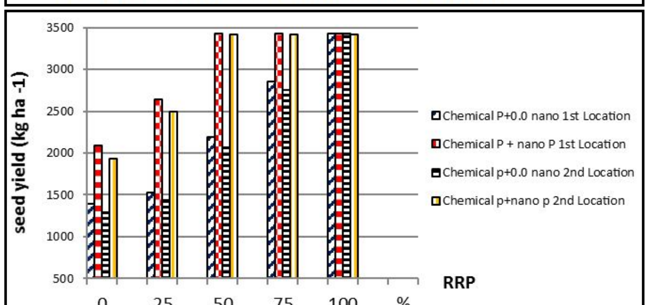

Fig.(8): Effect of the interaction between chemical and nano-phosphorus on seed yield

Table 3: Effect of chemical and Nano-fertilization of phosphorus zinc on straw yield (kg ha^{-1}) of soybean plant.

P		Zn (C)							
Chemical P	Nano rock P	1st Location				2nd Location			
		0.0	Sulphate	Nano	mean	0.0	Sulphate	Nano	Mean
0.0	0.0	1796.90	1961.10	1967.90	1908.63	1513.30	1675.70	1681.20	1623.4
	Nano	2120.40	2271.70	2282.30	2224.80	1667.90	1870.20	1876.90	1805.00
Mean		1958.65	2116.40	2125.10	2066.71	1590.60	1772.95	1779.05	1714.20
25%	0.0	1980.00	2136.90	2137.80	2084.90	1601.20	1706.90	1710.10	1672.73
	Nano	2305.20	2389.10	2390.80	2361.70	1889.50	2038.30	2044.30	1990.70
Mean		2142.60	2263.00	2264.30	2223.30	1745.35	1872.60	1877.20	1831.71
50 %	0.0	2193.80	2872.60	2880.00	2648.80	1776.40	1910.00	1911.70	1866.03
	Nano	3623.10	3792.10	3802.90	3739.36	3083.30	3342.10	3349.00	3258.13
Mean		2908.45	3332.35	3341.45	3194.08	2429.85	2626.05	2630.35	2562.08
75 %	0.0	3302.40	3479.90	3499.20	3427.16	2677.10	2950.00	2973.10	2866.73
	Nano	3621.20	3800.20	3812.60	3744.66	3073.50	3352.10	3356.00	3260.53
Mean		3461.80	3640.05	3655.90	3585.92	2875.30	3151.05	3164.55	3063.63
100 %	0.0	3621.60	3795.00	3801.00	3739.20	3070.40	3347.10	3353.80	3257.10
	Nano	3622.30	3805.70	3802.10	3743.36	3076.40	3355.10	3351.40	3260.96
Mean		3621.95	3800.35	3801.55	3741.28	3073.40	3351.10	3352.60	3259.03
mean of nano P	0.0	2578.94	2848.10	2857.18	2761.40	2127.68	1553.94	2352.98	2011.53
	Nano	3058.41	3211.96	3218.14	3162.83	2558.12	2791.56	2795.54	2715.07
mean of Zn		2818.67	3030.03	3037.66	2962.12	2342.90	2172.75	2574.62	2363.30
LSD at 5 %	A				24.5				26.7
	B				23.1				24.8
	C				19.3				20.5
	AB				27.5				28.3
	AC				NS				NS
	BC				NS				NS
	ABC				NS				NS

Effect of chemical and Nano-fertilization of phosphorus zinc on straw yield (kg ha^{-1}) of soybean plant

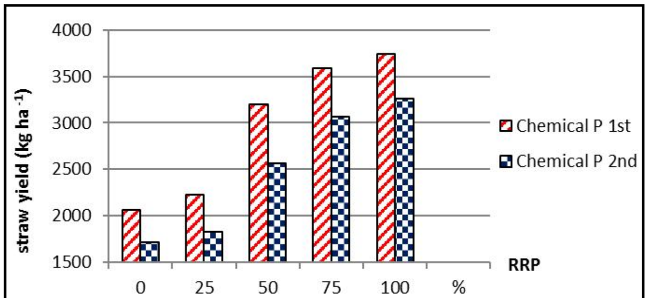

Fig.(9) : The main effect of chemical fertilization of phosphorus on straw yield

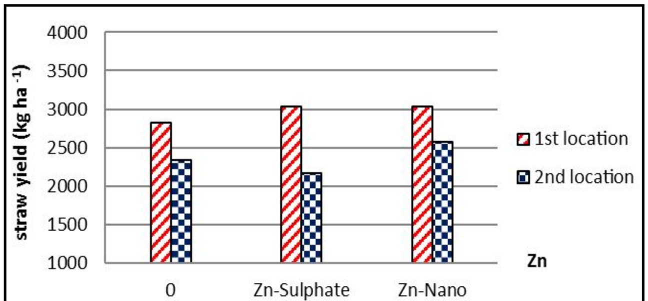

Fig.(11): The main effect of zinc on straw yield

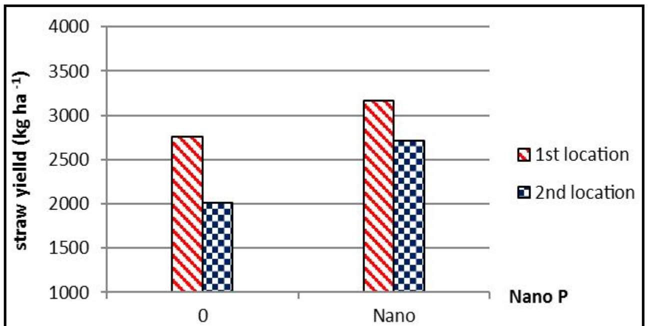

Fig.(10): The main effect of nano-phosphorus on straw yield

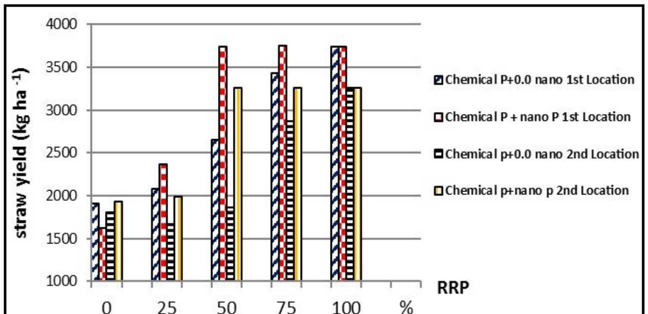

Fig.(12): Effect of interaction between chemical and nano-phosphorus on straw yield

about 5.6% in the first location. Same trends were obtained for the second location and straw yields. The beneficial effect of foliar spraying of zinc sulphate or Nano-zinc oxide may be due to its effect on improving enzymatic activity increasing photosynthesis and translocation of assimilates to seeds (Zeidan *et al.,* 2010). Moreover, (Awlad *et al.,* 2003) mentioned that the presence of Zn had positive effect on nodulation and symbiotic nitrogen fixation in legumes. (Benzon *et al.,* 2015) reported that Nano-zinc may have more effect on photosynthesis processes during its capability of translocation as its penetration and movement from roots and foliage of plant. These results agree with those obtained by (Thenua *et al.,* 2014 and Singh *et al.,* 2017) for zinc sulphate and (Adhikari *et al.,* 2016 and Dimkpa *et al.,* 2017) for zinc oxide Nanoparticles.

As for the interaction among the three factors, the results show that both seed and straw yields were affected only by the interaction between chemical (A) and Nano (B) fertilizers, where 50 % from phosphorus recommended rate under Nano-rock P produced grain and straw yields equal to those under 100% RR P. In general, combined 50% recommended rate of chemical phosphorus fertilizer with Nano-natural rock P plus foliar spraying of zinc sulphate or zinc oxide Nanoparticles exhibited the highest yields statistically equal to those under 100% chemical P fertilizers in both locations. On the other hand, the plants without each of chemical phosphorus fertilizer, natural rock P Nanoparticles or zinc oxide Nanoparticles exerted the lowest seed and straw yields. These results are in accordance with those obtained by (Shittu and Ogunwale 2012, Afra and Mozafar 2017) who reported that combined zinc with phosphorus fertilizer yielded the highest productivity of soybean.

Effect of different fertilization practices on protein content and yield

Data in tables 4 and 5 and illustrated in Figs. 13, 14, 15, 16, 17, 18, 19 and 20 show the effect of chemical P fertilizer, natural Nano rock P and zinc on protein percentage and total protein yield in soybean seeds. Concerning the main effect of chemical P fertilizer, the data indicate that both protein percentage and yield were significantly increased as phosphorus level increased up to 100% from its recommended rate. Protein percentage and yield resulted to add 100 % RRP were 18.5 and 131.8 % over control in the first location, respectively. The corresponding increases in the second location were 18.1 and 149.0 % in the above mentioned order. This increment may be due to more nitrogen fixation. In this concern, (Luikhan *et al.,* 2018) mentioned that phosphorus is an essential compound of DNA and may forms of RNA which needed for protein formation. These results agree with those obtained by (Win *et al.,* 2010 an Suman *et al.,* 2018).

Considering the main effect of Nano-natural rock, the data indicate that foliar spraying of rock P Nanoparticles increased both protein percentage and yield by about 7.1 and 38.7 % in the first location, respectively when compared with no rock P Nanoparticles. However, these increases in the second season were 7.2 and 41.4% as the same respect. The beneficial effects of rock P Nanoparticles is mainly explained by the greatest density in reactive area which enhanced its absorbed by plant similar to phosphorus from chemical fertilizer, hence enhanced N- fixation (Siddiqui *et al.,* 2015). These results are in harmony with those obtained by (Soliman *et al.,* 2016) on baobab plants.

Put the main effect of zinc in consideration, the results in tables 3 and 4 indicate that supplied soybean plant with zinc sulphate or zinc oxide Nanoparticles produced protein percent exceeded that produced under without zinc by about 3.9 and 4.0 %, respectively in the first location. The corresponding increasing in the protein yield were 9.7 and 10.0 % in the same order. Same trends were obtained in the second season.

The affirmative effect of Zn on increasing protein percentage and yield may be attributed to zinc application improved nitrogen metabolism, hence enhance the accumulation of amino acids and increased the rate of protein synthesis. In addition, Zn help to encourage more nodulation and leghemoglobin formation, consequently higher nitrogen and protein content. Also, zinc fertilization increased zinc in plant which enhance RNA and ribosome induction which accelerate protein synthesis (Dhanshree and Deshmukh, 2010 and Kulhare *et al.,* 2014). These results are similar to those obtained by (Kobraee and Shansi, 2015 and Raghuwanshi *et al.,* 2017). It is obvious to notice that the effects foliar application of zinc oxide Nanoparticles and zinc sulphate on protein percentage and yield were similar, which mainly due to the high mobility of Nano-particles or zinc sulphate, in turn ensures the phloem transport and ensures the nutrient to reach all plant organs (Gonzalez -Melendi *et al.,* 2008). These results are in harmony with those obtained by (Olkhovych *et al.,* 2016) for areca plant.

As for effect of the interaction, the data indicate that both protein percentage and yield of soybean seed were significantly affected by the interaction between chemical phosphorus and rock P Nanoparticles treatments (AXB), where combined 50% chemical P with rock P

Table 4: Effect of chemical and Nano-fertilization of phosphorus and zinc on protein (%) in seeds.

P		Zn (C)							
Chemical P	Nano rock P	1st Location				2nd Location			
		0.0	Sulphate	Nano	mean	0.0	Sulphate	Nano	Mean
0.0	0.0	31.31	33.13	33.13	32.52	31.25	33.00	32.93	32.39
	Nano	34.50	35.68	35.75	35.31	34.43	35.62	35.68	35.24
Mean		32.90	34.40	34.44	33.91	32.84	34.31	34.30	33.81
25%	0.0	33.50	34.87	34.93	34.43	33.37	34.81	34.87	34.35
	Nano	36.43	37.25	37.18	36.95	36.37	37.13	37.18	36.89
Mean		34.97	36.06	36.05	35.69	34.87	35.97	36.02	35.62
50 %	0.0	35.00	36.32	36.37	35.89	34.75	36.12	36.18	35.68
	Nano	38.93	40.62	40.68	40.07	38.75	40.50	40.56	39.94
Mean		36.97	38.47	38.53	37.98	36.75	38.31	38.37	38.81
75 %	0.0	36.32	37.00	37.06	36.79	36.25	36.87	36.93	36.68
	Nano	38.87	40.68	40.75	40.10	38.81	40.43	40.5	39.91
Mean		37.59	38.84	38.90	38.44	37.53	38.65	38.71	38.29
100 %	0.0	39.00	40.75	40.81	40.19	38.81	40.50	40.43	39.91
	Nano	39.06	40.75	40.75	40.19	38.87	40.43	40.50	39.93
Mean		39.03	40.75	40.78	40.19	38.84	40.47	40.47	39.92
mean of nano P	0.0	35.02	36.41	36.46	35.96	34.88	36.26	36.26	35.80
	Nano	37.55	38.99	39.02	38.52	37.44	38.82	38.88	38.38
mean of Zn		36.29	37.70	37.74	37.24	36.16	37.54	37.57	37.09
LSD at 5 %	A				1.03				1.01
	B				1.16				1.11
	C				0.96				0.89
	AB				1.38				1.35
	AC				NS				NS
	BC				NS				NS
	ABC				NS				NS

Effect of chemical and Nano-fertilization of phosphorus and zinc on protein (%) in seeds

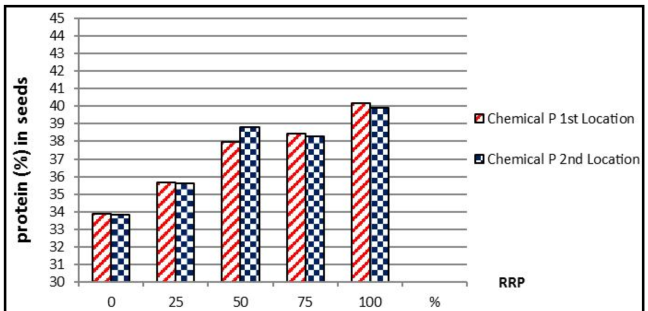

Fig.(13): The main effect of chemical fertilization of phosphorus on protien(%)

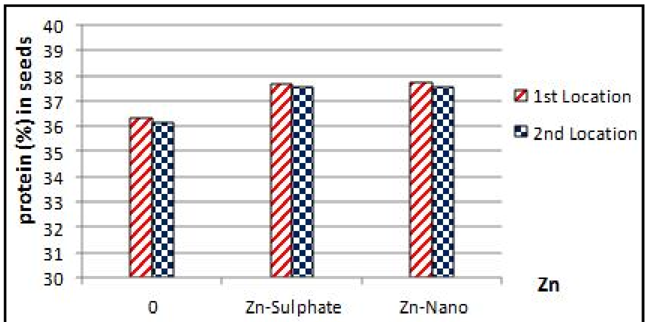

Fig.(15): The main effect of zinc on protein (%)

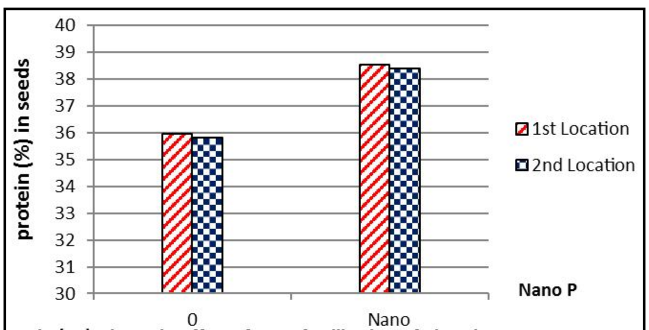

Fig.(14): The main effect of nano-fertilization of phosphorus on protein(%)

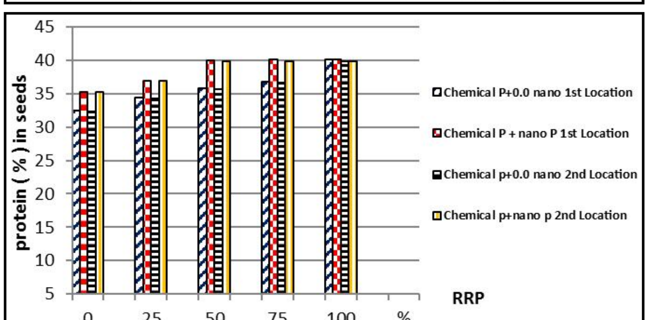

Fig.(16): Effect of the interaction between chemical and nano-phosphorus on protein (%)

Table 5: Effect of chemical and Nano-fertilization of phosphorus and zinc on protein yield (kg ha^{-1}).

P		Zn (C)							
Chemical P	Nano rock P	1st Location				2nd Location			
		0.0	Sulphate	Nano	mean	0.0	Sulphate	Nano	Mean
0.0	0.0	399.29	480.61	479.82	453.24	370.96	443.65	441.65	418.75
	Nano	679.33	766.01	767.58	737.64	624.28	706.48	708.74	679.83
Mean		539.31	623.31	623.70	595.44	497.62	575.06	575.19	549.29
25%	0.0	473.48	554.92	555.03	527.81	445.65	520.16	520.08	495.29
	Nano	926.26	1000.39	1000.27	975.64	865.46	946.29	948.12	919.95
Mean		699.87	777.66	777.65	751.72	655.55	733.22	734.10	707.62
50 %	0.0	720.40	822.17	822.79	788.45	680.26	764.91	766.72	737.29
	Nano	1310.42	1406.30	1408.78	1375.16	1245.72	1422.48	1426.37	1364.85
Mean		1015.41	1114.24	1115.78	1081.81	962.99	1093.70	1096.54	1051.07
75 %	0.0	998.18	1070.81	1080.08	1049.69	945.11	1041.72	1044.08	1010.30
	Nano	1309.68	1409.72	1412.19	1377.19	1255.58	1420.95	1424.62	1367.05
Mean		1153.93	1240.27	1246.13	1213.44	1100.34	1231.34	1234.35	1188.67
100 %	0.0	1312.54	1412.10	1414.22	1379.62	1257.40	1424.30	1421.88	1367.86
	Nano	1315.57	1412.47	1412.96	1380.33	1257.87	1421.19	1424.70	1367.92
Mean		1314.05	1412.29	1413.59	1379.98	1257.63	1422.75	1423.29	1367.89
mean of nano P	0.0	780.08	872.45	877.04	843.19	739.87	838.94	838.88	805.89
	Nano	1108.25	1198.90	1200.36	1169.17	1049.78	1183.47	1186.51	1139.92
mean of Zn		944.17	1035.68	1038.70	1006.18	894.82	1011.21	1012.69	972.90
LSD at 5 %	A				57.6				55.6
	B				61.3				60.2
	C				53.9				51.7
	AB				72.4				73.6
	AC				NS				NS
	BC				NS				NS
	ABC				NS				NS

Effect of chemical and Nano-fertilization of phosphorus and zinc on protein yield (kg ha^{-1})

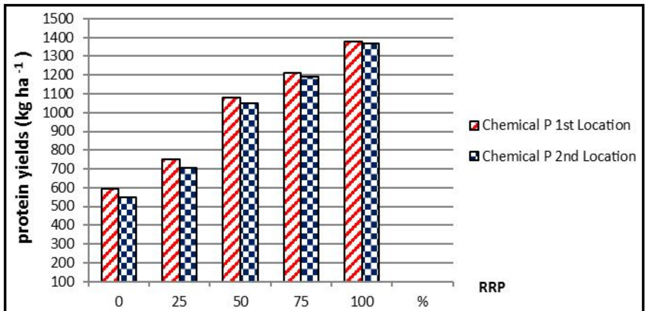

Fig.(17): The main effect of chemical fertilization of phosphorus on total protein yield

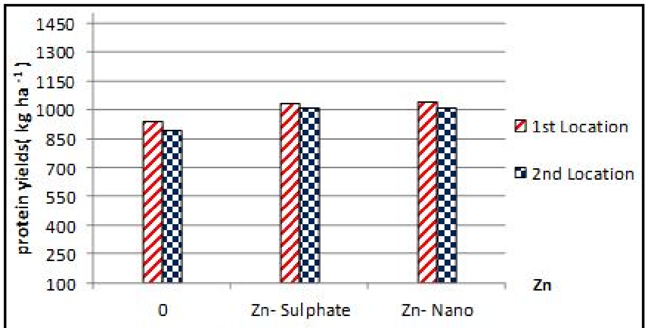

Fig.(19): The main effect of zinc on total protein yield

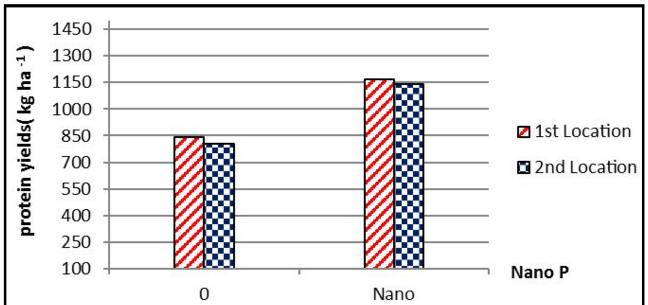

Fig.(18): The main effect of nano-fertilization of phosphorus on total protein yield

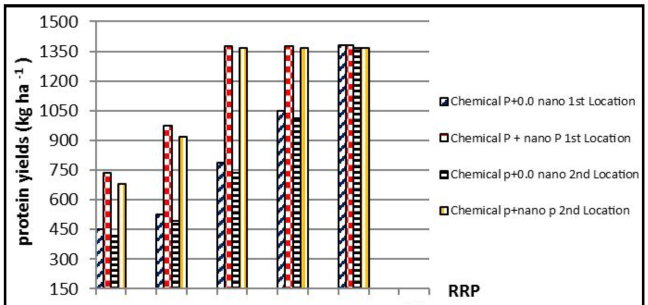

Fig.(20): Effect of the interaction between chemical and nano-phosphoruus on total protein yield

Table 6: Effect of chemical and Nano-fertilization of phosphorus and zinc on oil (%) in seeds.

P		Zn (C)							
Chemical P	Nano rock P	1st Location 0.0	Sulphate	Nano	mean	2nd Location 0.0	Sulphate	Nano	Mean
0.0	0.0	19.65	19.91	19.90	19.82	19.40	19.75	19.73	19.63
	Nano	20.85	21.05	21.07	20.99	20.66	20.96	20.95	20.86
Mean		20.25	20.48	20.48	20.40	20.03	20.35	20.34	20.24
25%	0.0	20.29	20.62	20.64	20.52	20.08	20.43	20.44	20.32
	Nano	21.17	21.55	21.53	21.42	21.00	21.34	21.33	21.22
Mean		20.73	21.08	21.08	20.97	20.54	20.88	20.88	20.77
50 %	0.0	20.96	21.30	21.33	21.19	20.74	21.23	21.23	21.06
	Nano	21.63	22.03	22.06	21.90	21.56	21.85	21.85	21.75
Mean		21.29	21.66	21.69	21.55	21.15	21.54	21.54	21.41
75 %	0.0	21.25	21.60	21.58	21.48	21.04	21.45	21.45	21.31
	Nano	21.61	22.01	22.04	21.88	21.55	21.86	21.86	21.75
Mean		21.43	21.81	21.81	21.68	21.29	21.65	21.65	21.53
100 %	0.0	21.64	22.02	22.05	21.90	21.56	21.87	21.87	21.77
	Nano	21.63	22.03	22.06	21.91	21.54	21.88	21.86	21.76
Mean		21.63	22.02	22.05	21.90	21.55	21.88	21.86	21.76
mean of nano P	0.0	20.75	21.09	21.10	20.98	20.56	20.94	20.94	20.81
	Nano	21.37	21.73	21.75	21.62	21.26	21.58	21.57	21.47
mean of Zn		21.06	21.40	21.42	21.29	20.91	21.26	21.25	21.14
LSD at 5 %	A				0.32				0.35
	B				0.21				0.24
	C				0.20				0.22
	AB				0.43				0.44
	AC				NS				NS
	BC				NS				NS
	ABC				NS				NS

Effect of chemical and Nano-fertilization of phosphorus and zinc on oil (%) in seeds

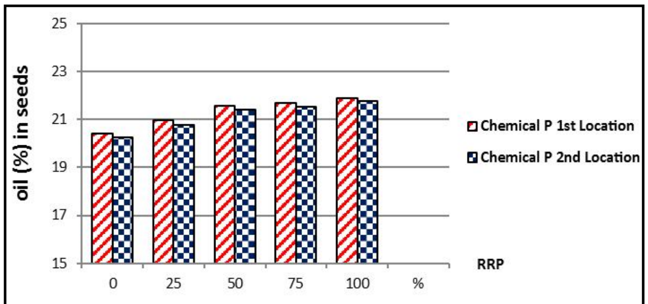

Fig.(21): The main effect of chemical fertilization of phosphorus on oil (%)

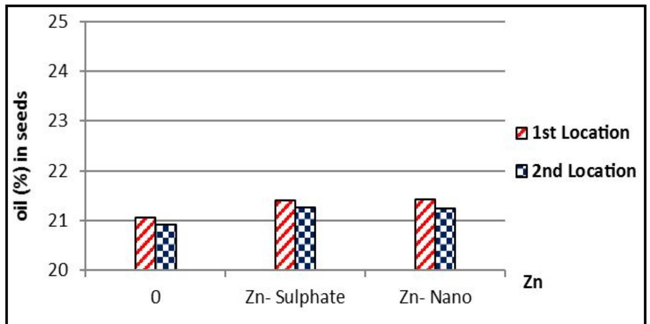

Fig.(23): The main effect of zinc on oil (%)

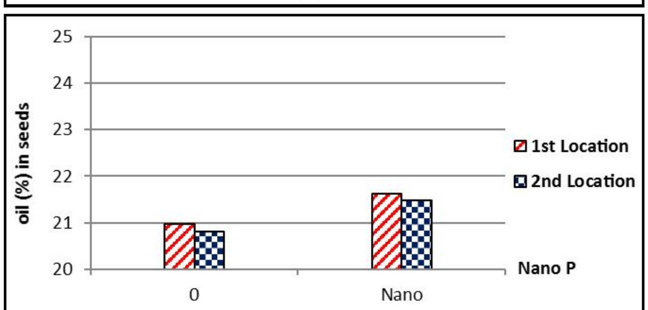

Fig.(22): The main effect of nano-fertilizzaatiionn of phosphorus on oil (%)

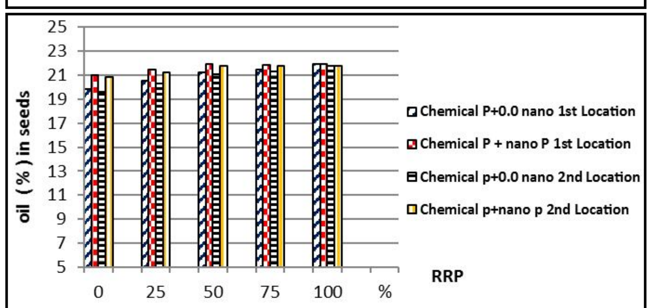

Fig.(24): Effect of the interaction between chemical and nano-phosphorus on oil (%)

Table 7: Effect of chemical and Nano-fertilization of phosphorus and zinc on oil yield (kg ha^{-1}).

P		Zn (C)							
Chemical P	Nano rock P	1st Location				2nd Location			
		0.0	Sulphate	Nano	mean	0.0	Sulphate	Nano	Mean
0.0	0.0	250.59	288.83	288.21	275.87	230.29	265.51	264.61	253.47
	Nano	410.55	451.92	452.39	438.28	374.60	415.72	416.15	402.15
Mean		330.57	370.38	370.30	357.08	302.44	340.61	340.38	327.31
25%	0.0	286.77	328.14	327.96	314.29	268.16	305.28	304.86	292.76
	Nano	538.26	580.83	580.68	566.59	499.71	543.87	543.93	529.17
Mean		412.51	454.48	454.32	440.44	383.93	424.57	424.39	410.96
50 %	0.0	431.41	482.16	482.54	465.37	406.00	449.37	449.90	435.09
	Nano	728.08	762.70	763.95	751.57	648.11	768.14	768.39	728.21
Mean		579.74	622.43	623.24	608.47	527.05	608.75	609.14	581.65
75 %	0.0	584.01	629.42	629.35	614.26	548.55	605.48	606.43	586.82
	Nano	728.12	762.73	763.79	751.54	697.18	768.29	768.94	744.80
Mean		656.06	696.07	696.57	682.90	622.86	686.88	687.68	665.81
100 %	0.0	728.21	763.05	764.12	751.79	698.52	769.12	769.14	745.59
	Nano	728.52	763.60	764.90	752.34	697.05	769.12	768.99	745.05
Mean		728.36	763.32	764.51	752.06	697.78	769.12	769.06	745.32
mean of nano P	0.0	456.21	498.32	484.32	484.32	430.30	478.95	478.98	462.74
	Nano	626.70	664.35	652.06	652.06	593.33	653.02	653.28	633.21
mean of Zn		541.45	581.33	581.78	568.19	511.81	565.98	566.13	547.97
LSD at 5 %	A				6.62				6.81
	B				5.19				5.37
	C				5.01				5.28
	AB				9.82				9.87
	AC				NS				NS
	BC				NS				NS
	ABC				NS				NS

Effect of chemical and Nano-fertilization of phosphorus and zinc on oil yield (kg ha^{-1})

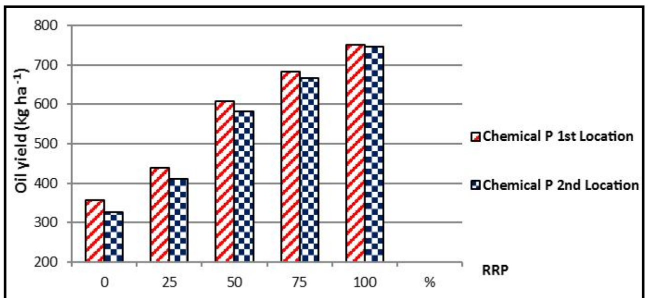

Fig.(25):The main effect of chemical fertilization of phosphorus on oil yield

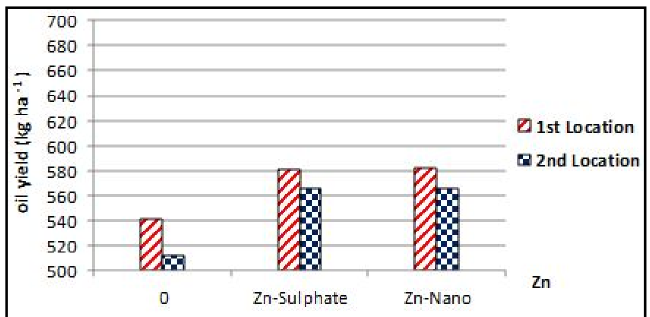

Fig.(27): The main effect of zinc on oil yield

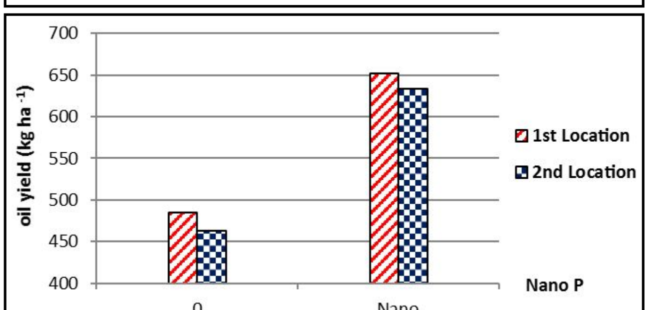

Fig.(26): The main effect of nano-fertilization of phosphorus on oil yield

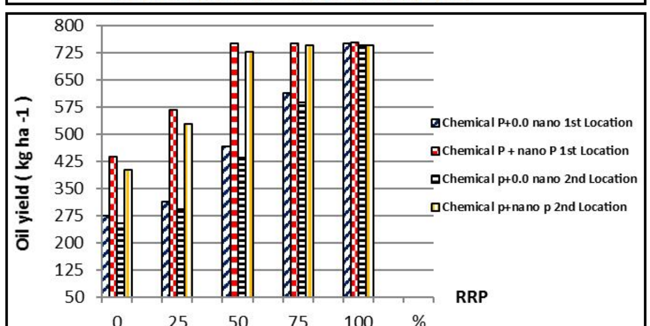

Fig.(28): Effect of the interaction between chemical and nano-phosphorus on oil yield

Nanoparticles produced protein percentage and yield equal to those under 100% chemical P. In general, the highest values of protein percentage and yield were achieved under the treatment of 100 % or 50 % chemical P + Nano-rock P + foliar spraying of $ZnSO_4$ or ZnO Nanoparticles; On the other hand, the plants without phosphorus fertilization and zinc application exhibited the lowest percent and yield of seed protein. These results are in agreement with those obtained by (Afra and Mozafar, 2017) who found a positive effect of combined phosphorus with zinc on increasing nitrogen content in soybean seed, consequently increased protein percentage and yield.

Effect of different fertilization practices on oil percentage and yield

The data in tables 6 and 7 and illustrated in Figs. 21, 22, 23, 24, 25, 26, 27 and 28 show the response of oil percentage or yield to chemical P, Nano-natural rock P and zinc whether sulphate salt or ZnO Nanoparticles. As for the main effect of chemical P fertilizer, the results reveal that oil percentage or yield increased as P level increased up to 100% from its recommended rate (RR). These increases due to 100% RR of P reached to 7.4 and 7.5 for oil (%) in the two studied locations, respectively. The corresponding increases for oil yield were 110.6 and 127.7 % in the above mentioned order. It is obvious to be observed that the increment due to P application was more pronounced for oil yield than oil percentage which mainly due to P application increased both yield table 2 and oil percentage table 6, since oil yield were determined by multiplying seed yield by oil percentage. The improvement of oil content in soybean seeds due to phosphorus application could be explained by the fact that phosphorus has an important role in fatty acids synthesis and their esterification by enhancing biochemical reactions in glyoxalase cycle (Dwiveddi and Bapat, 1998). These results agree with those obtained by (Win *et al.*, 2010 and Suman *et al.*, 2018).

With respect to the main effect of Nano-natural phosphorus rock, the results clearly indicate that foliar spraying of Nano-rock P increased both oil percentage and yield by about 3.1 and 34.6 %, respectively over no rock P application in the first location, while these increases in the second location were 3.2 and 36.8 % in the same respect. The affirmative effect of P as Nano-source could be due to its greater density in reactive area caused by its smaller particles, hence easily absorbed by plants, consequently increased oil content as mentioned before.

As for the main effect of zinc fertilization, the data reveal that both zinc sulphate and Nano-zinc oxide increased oil percentage by about 1.6 and 1.7 % in the first location comparing with no zinc in application and increased oil yield by about 7.4 and 7.4 % in the first location, respectively. Similar trends were obtained for the second location. Also, the data reveal that zinc oxide Nanoparticles has similar effect on oil content statistically equal to the effect of zinc sulphate. The affirmative effect of zinc on oil content is mainly due to activation of NADPH dependent dehydrogenase involved in fat formation by zinc application (Raghuwanshi *et al.*, 2017). These results were confirmed to those established by (Choudhary *et al.*, 2014 and Kuthare *et al.*, 2014).

As for the interaction effect, the data in tables 5 and 6 show that, oil percentage and yield were only affected by the interaction between chemical phosphorus fertilizer and natural rock P, where combined 50% RR chemical P with foliar spraying of Nano natural rock P gave oil content and yield statistically equal to those under 100% RR chemical P. In general, the highest oil percentage and yield were achieved by the treatment of 50% RR P + Nano-natural rock P + foliar spraying of Zn, whether sulphate salt or Nano-zinc oxide, or the treatment of 100% RR P with or without natural rock P plus zinc sulphate or zinc oxide. The beneficial effect of mixed phosphorus with zinc on oil content were reported by many workers such as (Afra and Mozafar 2017).

Conclusion

It could be concluded that supplied soybean plants with 50 % from phosphorus recommended rate with foliar spraying of 0.2 % twice Nano-natural rock P and foliar spraying of 0.2 % twice zinc sulphate solution or 0.2 % twice Nano-zinc oxide to obtain highest quality and quantity of soybean plant. This results means that it can save about 50 % from chemical phosphorus fertilizer by using Nano-rock P. Also, it can substitute zinc sulphate by use Nano-zinc oxide.

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