GROWTH AND DEVELOPMENT OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) VAR. PINTO SALTILLO EXPOSED TO IRON, TITANIUM, AND ZINC OXIDE NANOPARTICLES IN AN AGRICULTURAL SOIL

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(Received 29th Nov 2017; accepted 7th Mar 2018)

Abstract. Sustainable use of nanoparticles (NP) in the agriculture requires a deep understanding in order to determine their benefits potential as well as their toxicological impacts. Common bean plants were growing and irrigated with suspensions of magnetite, ferrihydrite, hematite, zinc oxide, or titanium dioxide NP at 0, 3, or 6 g L^{-1} in a 120 days' greenhouse experiment, in order to investigate the effect of these NP on growth and development of common bean. None of the five NP modified significantly the chlorophyll content of common bean plants, while at least one concentration of hematite, ferrihydrite or magnetite increased significantly the total N of roots or shoots, the number of pods, dry weight of pods, the number of seeds, and yield of common bean. Additionally, at least one concentration of zinc oxide or titanium dioxide decreased significantly the number of pods, the fresh weight of pods and the number of seeds. These finds are an important factor to take into account with regard to the applicability of NP for long-term use in crops, but the selection of the proper NP at their adequate concentration is important for realizing higher benefits for an agrosustainable target.
Keywords: *agro-food industry, agronanotechnology, chlorophyll content, potential hazard or risk of nanoparticles, sustainable development, nanofertilizer, Phaseolus vulgaris L.*

Introduction

While nanoparticles (NP) occur naturally in the environment and have been intentionally used for centuries, the production and use of engineered NP has seen a recent spike, which makes environmental release almost certain (Maurer-Jones et al., 2013). Keller et al. (2013) estimated that 63–91% of over 260,000–309,000 metric tons of global engineered nanomaterials production in 2010 ended up in landfills, with the balance released into soils (8–28%), water bodies (0.4–7%), and atmosphere (0.1–1.5%). It is well known that hundreds of NP are being used worldwide in a wide range

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_18831897

of products or devices, however, at our knowledge there are not standardized techniques nor laws governing the proper management of NP during their production, distribution, use, and confinement. This implies that NP may be released into the environment despite their potential harmful effects on human and environmental health.

Among some properties that comprise the bean are their high content of iron, vital for a proper brain development, help to correct biliary disorders, rheumatic diseases, lower cholesterol level and is effective against anemia, and their consumption can prevent some types of cancer. Per 100 common bean grams, 20 g are protein, 5.8 g are fat and 3 g are fiber (Lépiz et al., 2010).

In some prior research conducted on this topic, the results were varied, some of them showed favorable aspects due to the use of nanoparticles as the case of Ma et al. (2010), whose experimental data evinced that the TiO_2 nanoparticles at concentrations of 2.5-40 g kg^{-1} soil, improved the growth of the spinach. Other results exhibited no significant effects, as presented by Doshi et al. (2008), where aluminum nanoparticles did not show a significant effect on common bean studies performed in sand columns with concentrations up to 17 mg L^{-1} of aluminum. In addition, some studies present negative effects on the development and growth of established crops. Canas et al. (2008) indicated that monolayer carbon nanotubes caused significant affectations in the root elongation of crops such as tomato, cabbage, carrot and lettuce.

There have been published some studies that intend to demonstrate that some metallic NP are able to increase the growth and development of some crops, i.e., the NP are used as if they were fertilizers (Burke et al., 2015; Rico et al., 2011). However, the potential effect of NP on yield and yield components has not been studied when NP are considered as a collateral consequence of the NP polluted environment. It is still a challenge assessing most of the effect of NP in natural soils. It should be noted that NP have different pathways, effects, fates and behaviors that might vary within living organisms, soils and contaminants (Cornelis et al., 2014; Rodrigues et al., 2016). In this regard, the objective of this research was to investigate the effect of different nanoparticles such as iron, titanium and zinc oxide on growth and development of common bean plants cropped in an agricultural soil under greenhouse conditions, as a contribution to the new emerging field called econanotoxicology.

Materials and methods

Experimental site

This study was carried out in a greenhouse of the 'Programa de Sustentabilidad de los Recursos Naturales y Energía del Cinvestav-Saltillo' located in Saltillo, Coahuila, Mexico. This area is located in the southeastern state of Coahuila, centered at 25° 31' N, 101° 37' W, at an altitude of 1,600 m above sea level. According to FAO/UNESCO soil classification system, the soil is a Haplic Xerosol with pH 7.3 and electrolytic conductivity 4.8 dS m^{-1}, a water holding capacity (WHC) of 865 g kg^{-1}, an organic carbon content of 1.5 g C kg^{-1} soil, and a total N content of 0.7 g N kg^{-1} soil.

Biological material

Common bean seeds were donated by 'INIFAP-Celaya, Mexico'. All seeds were kept in the dark at 4 °C until use. Pinto Saltillo was developed to solve the problem of

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traditional varieties of ‘pinto’ type, which has a reduced postharvest life, due to the accelerated darkening of the seed coat.

Nanomaterials

Nanoparticles of magnetite, ferrihydrite and hematite were manufactured according to Pariona (2012), while nanoparticles of zinc oxide and titanium dioxide were purchased from ‘Materiales nanoestructurados S.A de C.V. (San Luis Potosí, México)’. Its crystallographic system was cubic, hexagonal or tetragonal (*Table 1*). The X-ray diffraction was conducted to verify the pure phase samples (*Fig. 1*), and the magnetic properties of the samples were measured by MicroMagTM 2900 Alternating Gradient Magnetometer (*Fig. 2*).

Table 1. *Physicochemical characteristics of nanoparticles used to irrigate common bean crop (Phaseolus vulgaris* L.*) in a 120 days greenhouse experiment.*

Oxide	Molecular formula	Color	Particle size	Crystallographic system	Magnetic properties
Magnetite	Fe_3O_4	Black	6 a 20 nm	Cubic	Superparamagnetic
Ferrihydrite	$FeOOH \bullet xH_2O$	Dark brown	2 a 3 nm	Hexagonal	Antiferromagnetic
Hematite	α-Fe_2O_3	Red ochre	80 a 94 nm	Hexagonal	Weakly antiferromagnetic
Zinc oxide	ZnO	White	< 50 nm	Tetragonal	Weakly ferromagnetic
Titanium dioxide	TiO_2	White	< 50 nm	Hexagonal	Weakly ferromagnetic

Cultivation of plants in the greenhouse

The full experimental setup was repeated three times. The first one was carried out from January to May, 2016, the second one, from February to June, 2016, and the third one from March to July, 2016. Sixty sub-samples of 3,500 g soil, i.e., five kinds of nanoparticles (nano-Fe_3O_4, nano-$FeOOH \bullet xH_2O$, nano-α-Fe_2O_3, nano-ZnO, and nano-TiO_2) × three replicates × four concentrations, were added to square plastic pots whose length, width, and height were 17 × 15 × 17 cm, respectively. Five treatments (nanoparticles) at four concentrations (zero, one, three, and six g L^{-1}) were applied to the soil during irrigation so that we sprayed each plastic pot with 500 mL of a zero, one, three, or six g nano L^{-1} suspension, throughout the experiment. Three seeds of common bean were planted in one hundred and eighty plastic pots, i.e., five nanoparticles × three replicates × four concentrations on three experiments. The seeds were placed at two cm depth in each plastic pot. Five days after planting, the seedlings were thinned to one plant per plastic pot. The plastic pots were placed in the greenhouse for 120 days. A plastic container was placed under each plastic pot to collect drained liquid. However, irrigation was well controlled so that no leaching was observed. Thirty, 60 and 120 days after sowing, three plastic pots were selected at random from each treatment and each concentration. The entire soil column was removed from the plastic pot and the 0-7.5 cm and 7.5-15 cm depth, where the samples were taken with care not to damage the root structure. The roots were manually separated from the shoots of the plant. Then the soil was carefully disaggregated with the hands to avoid the rupture of the roots. Subsequently, the soil was sifted gently to extract the pieces of root that may have been left in it, in order to be sure that 100% of the roots were removed, washed and weighed. After that, the root was extended and measured along with the shoots length. The roots

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_18831897

and shoots were dried at 70 °C, were weighed and analyzed for Ti, Fe, Zn, and total N. The soils from 0-7.5 cm and 7.5-15 cm of depth were analyzed for pH, CE, Ti, Fe, and Zn. The amount of chlorophyll was quantified every two days after sowing, beginning at day 15 (*Fig. 3*). The temperature and moisture content inside the greenhouse during the experiment were 24 °C and 35-45%, respectively.

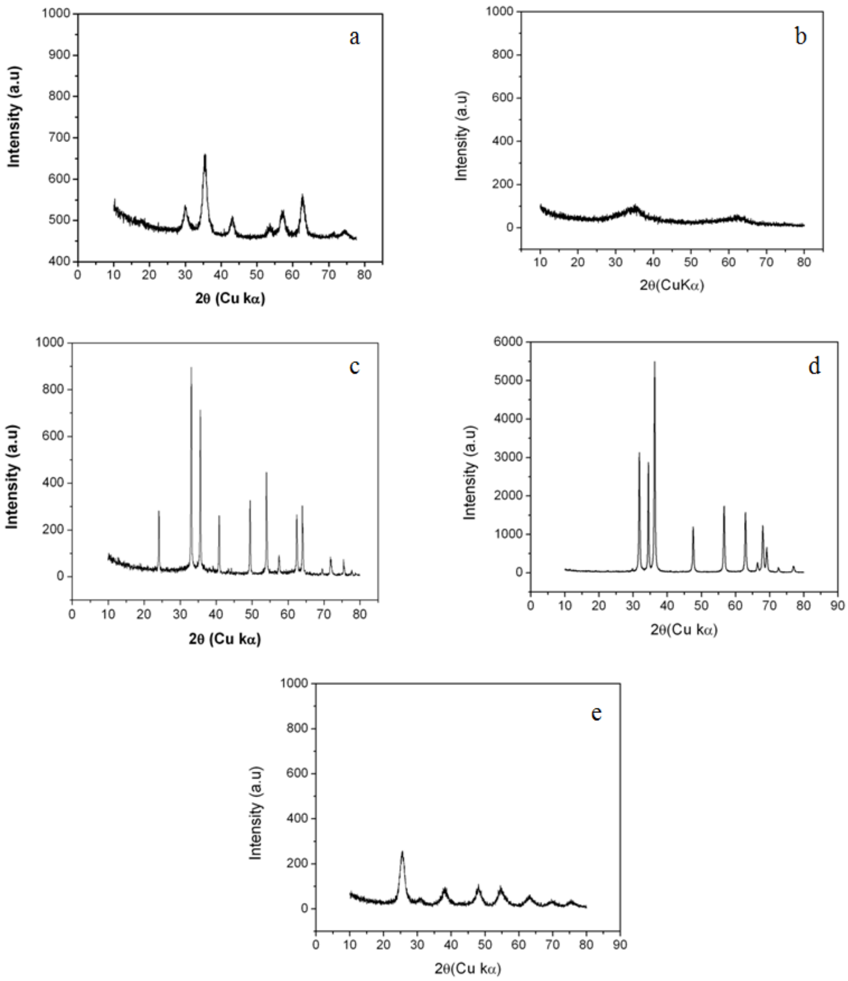

Figure 1. *X-ray diffraction patterns of nanoparticles of a) Magnetite, b) Ferrihydrite, c) Hematite, d) ZnO, and e)* TiO_2.

Chemical analyses

The pH was measured in 1:2.5 soil or wastewater sludge/H_2O suspension using a 716 DMS Titrino pH meter (Metrohm Ltd. CH.-901, Herisau, Switzerland) fitted with a glass electrode (Thomas, 1996). The EC was determined in a 1:5 soil/H_2O suspension as described by Rhoades et al. (1989). The organic C in soil was measured in a total organic carbon analyzer TOC-VCSN (SHIMADZU, USA). The inorganic C was determined by adding 5 mL 1 M hydrogen chloride (HCl) solution to 1 g air-dried soil and trapping CO_2 evolved in 20 mL 1 M NaOH. Total N in soil, root and shoot was measured by the Kjeldahl method using concentrated H_2SO_4, K_2SO_4 and $CuSO_4$ to digest the sample (Bremner, 1996). Soil particle size distribution was defined by the hydrometer method as described by Gee and Bauder (1986). Water holding capacity

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was measured on 6.5 kg soil placed in a PVC tube (length 50 cm and ∅ 16 cm), water-saturated, stoppered with a PVC ring and left to stand overnight to drain freely. The WHC is defined as (Gardner, 1986), WHC = [(soil water-saturated - soil dried at 105 °C) / soil dried at 105 °C] * 1000. The units of WHC are expressed in g kg^{-1}. The amount of chlorophyll was measured with a Minolta SPAD-502 Chorophyll meter (Markwell et al., 1995). The Fe, Ti and Zn were determined by inductively coupled plasma mass spectrometry (ICP–MS).

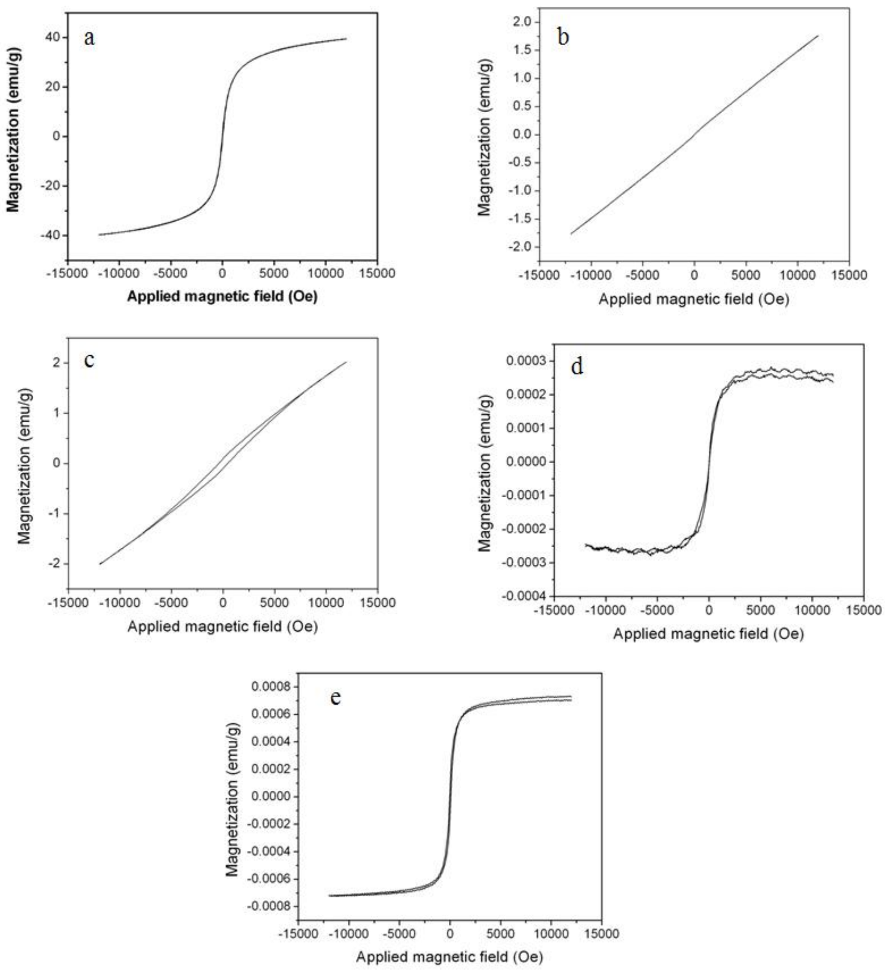

Figure 2. *Magnetization curves of a) Magnetite, b) Ferrihydrite, c) Hematite, d) ZnO and e) TiO_2.*

Statistical analyses

The data were subjected to an analysis of variance (ANOVA) and means compared with the Tukey test using Statistical Analysis System (SAS) software version 8.0 for Windows (SAS Institute, 1989). Soil and plant characteristics were subjected to one-way analysis of variance using a general lineal models procedure (PROC GLM) to test for significant differences between treatments ($P < 0.05$). Methodology for PCA analysis may be found in Fernández-Luqueño et al. (2016). All analyses were performed using the SAS statistical package (SAS Institute, 1989). All data presented

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1602_18831897

were the mean of three replicates in soil from three different plots, while the whole experiment was repeated three times (n = 27), sampled after 30, 60, and 120 days.

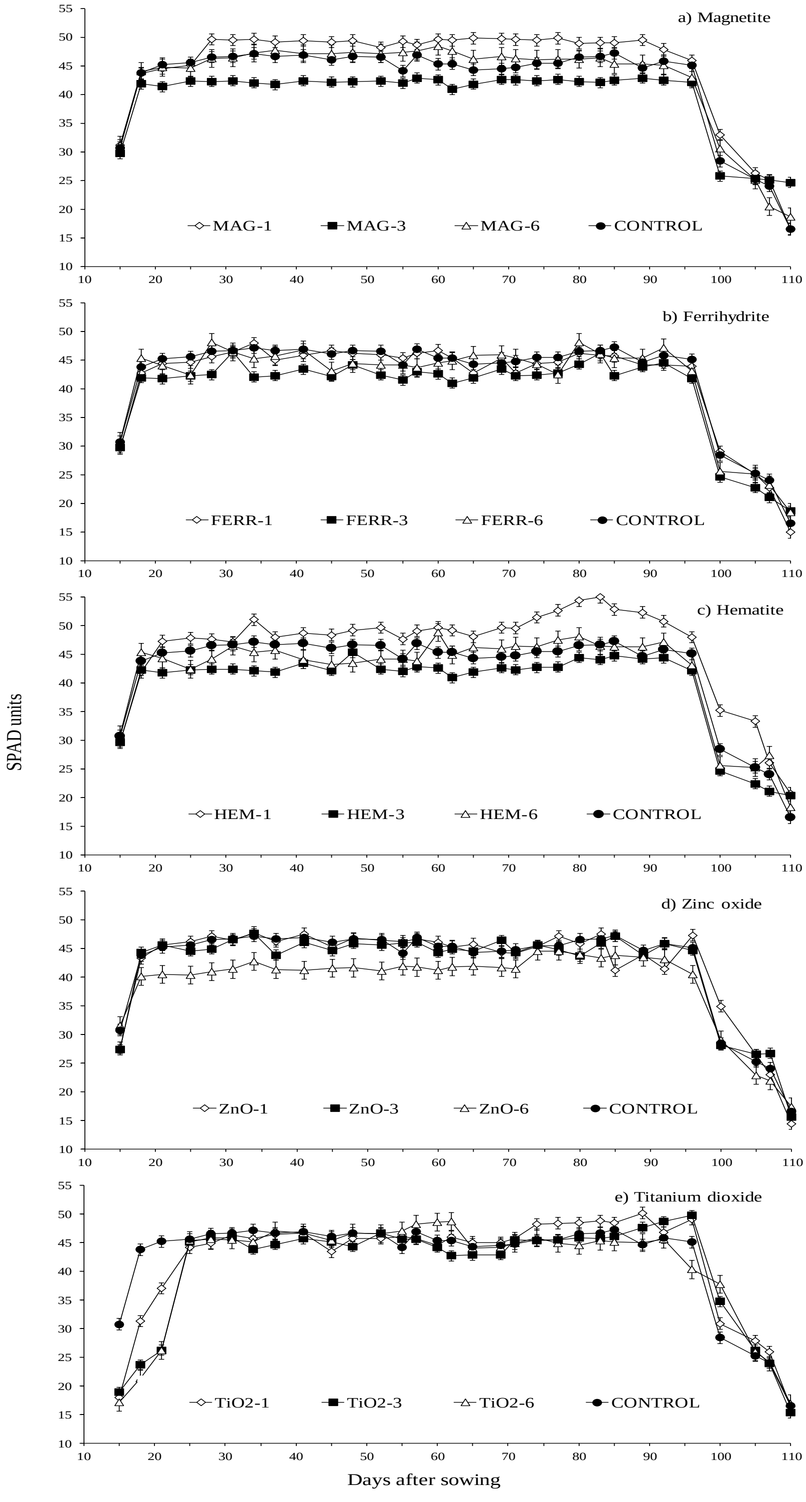

Figure 3. *SPAD units of bean plants (Phaseolus vulgaris L.) cultivated in an agricultural soil irrigated with 500 mL of zero, one, three, or six g nanoparticle* L^{-1} *suspension. Nanoparticles of* Fe_3O_4*,* $FeOOH{\bullet}xH_2O$*,* $\alpha\text{-}Fe_2O_3$*, ZnO, and* TiO_2 *were used. Data are the mean of three pots × three different soils × three experiments × three measurements by each plant, i.e., n = 81. Each experiment lasted 120 days.*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Results

SPAD units

The concentration of the chlorophyll quantified on leaves of common bean plants cultivated in an agricultural soil irrigated with 500 mL of zero, one, three, or six g nanoparticle L^{-1} suspension, during 120 days after sowing is shown in *Figure 3*. SPAD Units data are related to the chlorophyll content, which maintained slightly unchanged over the time with values between 40 to 50 SPAD units in most of the experiment, excepting the HEMATITE treatment (HEM-1) which the SPAD units reached 55. The lowers chlorophyll values were presented in all the treatments during the onset of the measurements and at the end of the growing stage, i.e., at 15 and 95 days after sowing, respectively.

Plant characteristics and crop yield

Most of the plant and yield characteristics (root fresh weight, root dry weight, root length, shoot fresh weight, shoot dry weight, shoot length, and SPAD units) were not significantly different between nanoparticles treatments, compared with the CONTROL treatment ($P < 0.05$) (*Table 2*). FERRIHYDRITE treatments at 3 and 6 g L^{-1} and HEMATITE at 1 g L^{-1} increased significantly the concentration of total N in roots, compared with the CONTROL treatment. All FERRIHYDRITE treatments and HEMATITE at 3 and 6 g L^{-1} increased significantly the concentration of total N in shoots, compared with the CONTROL treatment (*Table 2*).

The MAGNETITE and FERRIHYDRITE treatments at 6 g L^{-1} increased significantly the number of pods, compared with the CONTROL treatment. However, the ZINC OXIDE and TITANIUM OXIDE treatments at 6 g L^{-1} decreased significantly the number of pods, compared with the CONTROL treatment (*Table 2*). The fresh weight of pods decreased significantly when plants were amended with MAGNENTITA or HEMATITE at 3 or 6 g L^{-1}, and when they were amended with FERRIHYDRITE or ZINC OXIDE at 6 g L^{-1}, compared with the CONTROL treatment (*Table 2*). The dry weight of pods decreased significantly when plants were amended with FERRYDRITE at 3 g L^{-1}, but MAGNETITE at 3 or 6 g L^{-1} increased significantly the dry weight of pods, compared with the CONTROL treatment (*Table 2*). MAGNETITE and FERRIHYDRITE increased significantly the number of seeds when plants were amended with 6 g L^{-1}, while HEMATITE increased it significantly when plants were amended with 3 or 6 g L^{-1}. However, plants amended with ZINC OXIDE or TITANIUM DIOXIDE decreased significantly the number of seeds, compared to the CONTROL treatment. MAGNETITE or HEMATITE at 3 or 6 g L^{-1} and FERRIHYDRITE at 6 g L^{-1} increased significantly the yield, while ZINC OXIDE or TITANIUM DIOXIDE decreased it significantly, compared with the CONTROL treatment (*Table 2*). Nanoparticles did not affect significantly the SPAD units.

The seed number was strongly significantly correlated with fresh or dry weight of roots, length of root or shoot, total nitrogen of shoot, and with number, fresh or dry weight of pods (*Table 3*). Seed yield was strongly significantly correlated with fresh weight of root, total nitrogen of root, dry weight of shoot, total nitrogen of shoot, with number, fresh or dry weight of pods, and with seed number. In addition, SPAD units was strongly significantly correlated with total nitrogen of shoot and with yield (*Table 3*).

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Table 2. *Characteristics of common bean (Phaseolus vulgaris* L.*) cultivated in an agricultural soil irrigated with 500 mL at zero, one, three, or six g nanoparticle L^{-1} suspension. The whole experiment was repeated three times (the first time, from January to May, 2016; the second one, from February to June, 2016; and the third one, from March to July, 2016). Each whole experiment lasted 120 days. Root and shoot data are the mean of values measured after 30, 60 and 120 d, i.e., n = 81. The pods and seeds data are the mean of values measured after 120 d, i.e., n = 27. SPAD unit's data are the mean of three measures twice a week during 14 weeks, i.e., n = 1,988 (3 soils × 3 replicates × 3 measures per plant × 3 sampling dates × 2 measures per week × 14 weeks).*

Treatments / g NP L^{-1}	**Root [Ø]**				**Shoot**				**Pods**			**Seeds**		**SPAD**
	Fresh weight	**Dry weight**	**Length**	**Total Nitrogen**	**Fresh weight**	**Dry weight**	**Length**	**Total Nitrogen**	**Number**	**Fresh weight**	**Dry weight**	**Number**	**Yield**	**Units**
Control														
0	13.6 a[Σ]	1.1 a	28.1 ab	25.8 c	17.9 a	2.8 a	21.4 ab	27.3 e	14.6 cd	20.5 de	5.5 cd	49.6 cd	3.6 fg	35.8 ab
Magnetite (Fe_3O_4)														
1	10.0 a	1.0 a	24.9 ab	26.4 bc	15.6 a	1.7 a	22.0 ab	30.3 cde	15.7 bcd	20.9 cde	5.9 c	48.7 cde	3.9 defg	38.4 a
3	11.3 a	0.8 a	24.0 ab	28.9 abc	14.5 a	1.5 a	22.7 ab	29.8 cde	16.3 bc	22.5 bc	6.6 ab	49.0 cde	4.8 ab	35.8 ab
6	13.5 a	1.2 a	19.0 ab	30.4 abc	17.3 a	1.9 a	25.9 b	32.1 bcde	17.3 ab	26.0 a	7.1 a	64.3 a	4.7 bc	36.4 ab
Ferrihydrite ($FeOOH•xH_2O$)														
1	11.8 a	1.0 a	28.9 ab	30.5 abc	17.8 a	2.6 a	21.4 ab	33.8 abcd	14.7 cd	20.7 cde	5.5 cd	55.0 bc	3.6 g	36.0 ab
3	11.6 a	1.1 a	27.8 ab	32.1 a	16.7 a	2.9 a	21.9 ab	34.9 abc	15.3 bcd	20.3 cd	4.5 e	51.0 cd	3.8 efg	36.0 ab
6	12.3 a	1.3 a	27.1 ab	32.0 a	15.7 a	2.7 a	19.2 ab	35.0 abc	19.3 a	26.9 a	5.6 cd	67.0 a	4.2 cd	36.6 ab
Hematite (α-Fe_2O_3)														
1	14.7 a	1.3 a	25.1 a	31.0 ab	18.3 a	3.0 a	20.2 ab	32.3 bcde	15.7 bcd	22.0 bcd	5.5 cd	53.7 bcd	4.0 def	37.6 ab
3	14.3 a	1.3 a	28.2 ab	30.0 abc	19.5 a	3.2 a	17.0 ab	37.2 ab	15.0 cd	23.6 b	5.9 c	60.0 ab	5.2 a	35.7 ab
6	14.8 a	1.4 a	31.0 ab	30.0 abc	18.1 a	2.9 a	19.4 ab	38.5 a	16.0 bcd	25.9 a	5.9 bc	60.7 ab	5.1 a	36.6 ab
Zinc Oxide (ZnO)														
1	11.1 a	0.9 a	21.2 ab	28.1 abc	15.7 a	2.4 a	17.0 ab	29.8 cde	15.0 cd	19.4 e	5.2 cde	45.3 def	4.0 defg	35.3 ab
3	11.9 a	1.0 a	19.6 ab	29.1 abc	15.5 a	2.2 a	15.7 a	30.2 cde	15.3 bcd	21.1 cde	5.5 cd	40.7 ef	3.9 defg	34.9 ab
6	13.1 a	0.8 a	21.1 ab	28.6 abc	16.8 a	2.6 a	18.2 ab	30.4 cde	12.3 e	23.8 b	5.2 cde	37.7 fg	4.1 de	36.7 ab
Titanium dioxide (TiO_2)														
1	11.1 a	0.9 a	17.6 ab	27.7 abc	13.8 a	2.1 a	17.5 ab	28.3 e	14.0 de	19.2 e	5.0 de	31.3 gh	4.0 defg	34.7 b
3	11.9 a	1.0 a	17.1 b	30.0 abc	13.3 a	2.4 a	15.7 a	28.8 de	15.7 bcd	21.1 de	5.3 cd	23.7 h	3.9 defg	35.7 ab
6	11.5 a	1.0 a	22.0 ab	28.5 abc	18.2 a	2.0 a	16.3 a	29.5 de	12.0 e	21.9 bcd	5.0 de	26.3 h	4.1 de	34.2 b
MSD[π]	9.05	0.64	13.5	5.0	9.4	1.7	9.2	5.4	2.0	1.9	0.7	8.5	0.4	3.5

[Ø]Fresh or dry weights are expressed in g; Length is in cm; Total nitrogen is in g N kg^{-1} dry plant; Yield is in g per plant.
[Σ]Values with the same letter within the columns are not significantly different ($P < 0.05$).
[π]Minimum significant difference ($P < 0.05$).

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Table 3. *Correlations between characteristics of common bean crop (Phaseolus vulgaris* L.*) cultivated in an agricultural soil irrigated with 500 mL at zero, one, three, or six g NP L^{-1} suspension. Data were pooled among five treatments, three soils and the three replicates of the whole experiment. Each experiment lasted 120 days.*

Plant characteristics	**Root^{Ø}**				**Shoot**				**Pods**			**Seeds**	
	Fresh weight	**Dry weight**	**Length**	**Total Nitrogen**	**Fresh weight**	**Dry weight**	**Length**	**Total Nitrogen**	**Number**	**Fresh weight**	**Dry weight**	**Number**	**Yield**
Root dry weight	0.867***												
Root length	0.891***	0.834***											
Root total N	-0.238***	0.005	-0.289***										
Shoot fresh weight	0.839***	0.870***	0.762***	0.142									
Shoot dry weight	0.894***	0.876***	0.852***	-0.062	0.883***								
Shoot length	0.848***	0.815***	0.809***	-0.075	0.837***	0.780***							
Shoot Total N	-0.037	0.245***	-0.041	0.839***	0.313***	0.150	0.121						
Pods number	0.279	0.428***	0.210	0.155	-0.064	-0.108	0.297***	0.166					
Pods fresh weight	0.344***	0.462***	0.155	0.332***	-0.278	-0.121	0.078	0.501***	0.292***				
Pods dry weight	0.062	0.130***	0.023	0.005	-0.218	-0.429***	0.379***	0.112	0.272	0.341***			
Seed number	0.415***	0.616***	0.647***	0.058	0.115	0.195	0.492***	0.421***	0.480***	0.400***	0.343***		
Seed yield	0.200***	0.241	0.060	0.396***	-0.183	-0.301***	-0.013	0.622***	0.276**	0.475***	0.295***	0.270***	
SPAD Units	-0.039	0.001	-0.008	0.099	-0.014	-0.017	0.023	0.186***	0.182	0.122*	0.121	0.149	0.341***

*P < 0.005; **P < 0.001; ***P < 0.0001

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Principal component analysis

Loading for parameters obtained after VARIMAX rotation are given in *Table 4*. The plants characteristics had three significant PCs. The first principal component (PC1) explained 31% of variation and was related to root fresh weight, root dry weight, root length, shoot total nitrogen, number of pods, pod fresh weight, seed yield, number of seeds, and SPAD units. The second principal component (PC2) explained 20% of variation and was related to shoot fresh weight and shoot dry weight, while the third principal component (PC3) explained 14% of variation and was related to root total nitrogen but negatively related to shoot length and pod dry weight. The three principal components explained 65% of variation (*Table 4*).

On the scatter plot with PC1 and PC2, the kinds of NP or their concentrations are clearly separated from each other (*Fig. 4a*). HEMATITE and FERRIHYDRITE can be found in the upper right quadrant, while MAGNETITE, ZINC OXIDE or TITANIUM DIOXIDE lie in the two left quadrants. The CONTROL treatment lies in the lower left quadrant (*Fig. 4a*). On the scatter plot with PC1 and PC3, the treatments are visually distinct (*Fig. 4b*). The HEMATITE, FERRIHYDRITE, and CONTROL treatments lie in the two-right quadrant, while MAGNETITE, ZINC OXIDE or TITANIUM DIOXIDE lie in the two left quadrants (*Fig. 4b*).

Table 4. *Rotated loading on the PC of bean plants characteristics (Phaseolus vulgaris* L.*) cultivated in an agricultural soil irrigated with 500 mL at zero, one, three, or six g NP L^{-1} suspension. NP of Fe_3O_4, $FeOOH{\bullet}xH_2O$, α-Fe_2O_3, ZnO, and TiO_2 were used. Data were pooled among the five treatments and three experiment repetitions. The whole experiment was repeated three times (from January to May 2015; the second one from February to June 2015; and the third one from March to July 2015). Each whole experiment lasted 120 days.*

Statistical and measurements	**Principal components** [a]		
	PC1	**PC2**	**PC3**
Eigenvalues	4.27	2.83	1.94
Proportions	0.31	0.20	0.14
	Rotated loading on three retained components		
Root fresh weight	65*,[b]	14	30
Root dry weight	80*	32	5
Root length	67*	61	-9
Shoot fresh weight	20	74*	10
Shoot dry weight	24	79*	42
Shoot length	35	12	-80*
Root total nitrogen	33	-50	59*
Shoot total nitrogen	70*	-31	44
Number of pods	57*	-10	-26
Pod fresh weight	64*	-40	6
Pod dry weight	36	-37	-54*
Seed yield	52*	-60	12
Number of seeds	80*	17	-29
SPAD units	43*	-31	-9

[a]Only principal components with Eigenvalues>1 and that explain > 10% the total variance were retained
[b]Parameters with significant loading (> 0.4) on the within column principal component.

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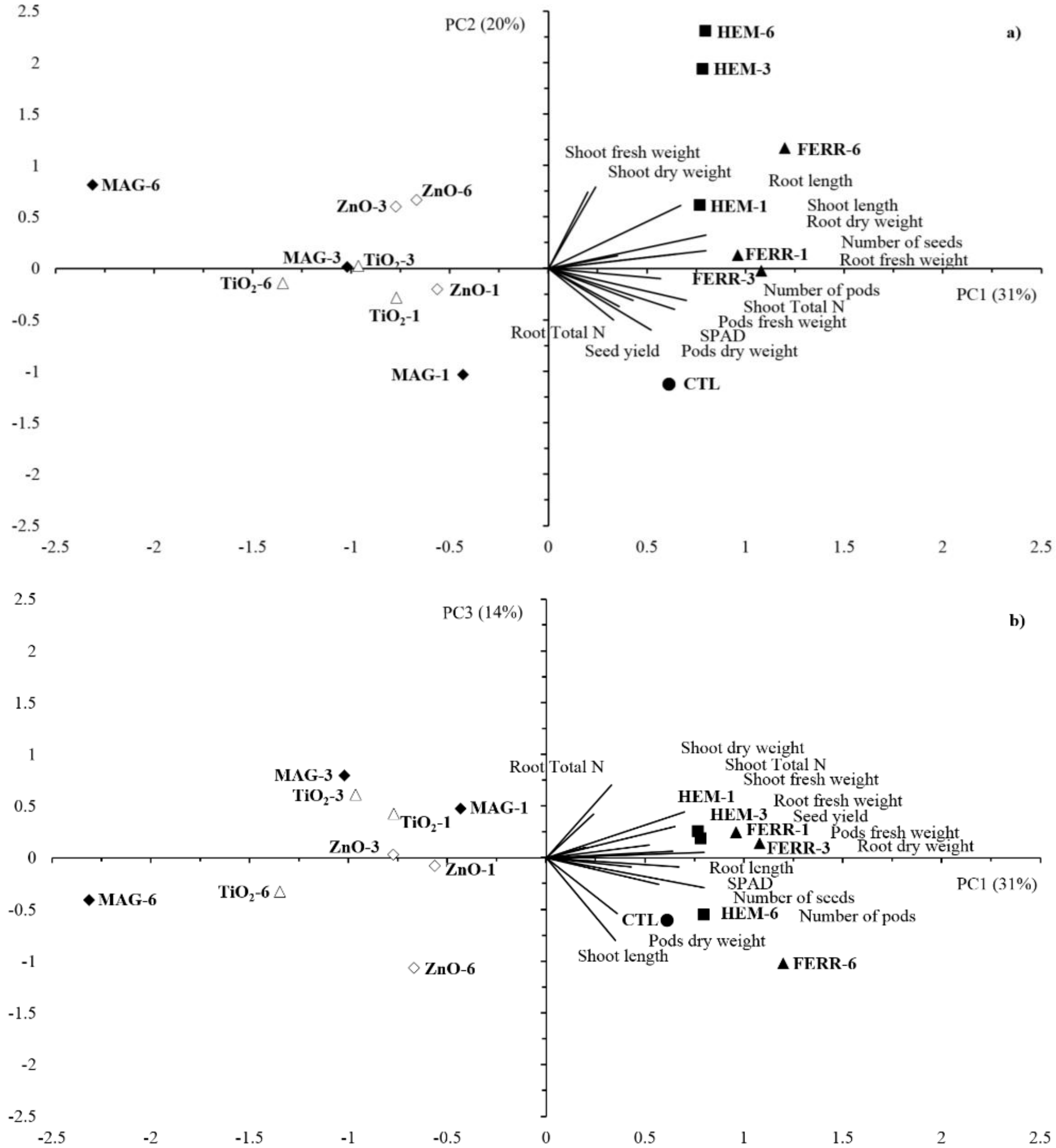

Figure 4. *Principal component analysis performed on characteristics of bean plants (Phaseolus vulgaris* L.*) cultivated in an agricultural soil irrigated with 500 mL of zero, one, three, or six g nanoparticle L^{-1} suspension. Nanoparticles of Fe_3O_4, FeOOH•xH_2O, α-Fe_2O_3, ZnO, and TiO_2 were used. Data are the mean of three square plastic pots with 3.5 kg dry soil each one, for three different soils and three experiments, i.e., n = 27. Each whole experiment lasted 120 days. The first two factors explain 51% of the variation. MAG-1 (500 mL of 1 g NP-Fe_3O_4 suspension), MAG-3 (500 mL of 3 g NP-Fe_3O_4 suspension), MAG-6 (500 mL of 6 g NP-Fe_3O_4 suspension); FERR-1 (500 mL of 1 g NP-FeOOH•xH_2O suspension), FERR-3 (500 mL of 3 g NP-FeOOH•xH_2O suspension), FERR-6 (500 mL of 6 g NP-FeOOH•xH_2O suspension); HEM-1 (500 mL of 1 g NP-α-Fe_2O_3 suspension), HEM-3 (500 mL of 3 g NP-α-Fe_2O_3 suspension), HEM-6 (500 mL of 6 g NP-α-Fe_2O_3 suspension); ZnO-1 (500 mL of 1 g NP-ZnO suspension), ZnO-3 (500 mL of 3 g NP-ZnO suspension), ZnO-6 (500 mL of 6 g NP-ZnO suspension); TiO_2-1 (500 mL of 1 g NP-TiO_2 suspension), TiO_2-3 (500 mL of 3 g NP-TiO_2 suspension), and TiO_2-6 (500 mL of 6 g NP-TiO_2 suspension).*

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Discussion

All treatments showed a lower chlorophyll content during the onset of the measurement at 15 days after sowing. This behavior suggests that the common bean plants were not in the fullness of the photosynthetic process. SPADS curves commonly show a decreasing trend due to the measurement was done leaf by leaf at the beginning of vegetative growth (Ribeiro de Cunha et al., 2015). About the SPAD units, Gómez et al. (2011) reported values near to 40 – 45 SPAD units. Anderson and Ryser (2015). However, measured leaves chlorophyll concentration in common bean reported range between 30 and 40 SPAD units. In this study, none of the treatments increased the chlorophyll content of bean plants, nevertheless the SPAD units' values are consistent with the values reported by other authors. Fernández-Luqueño et al. (2008, 2010) stated that the SPAD units decreased abrupt and significantly as soon as onset the plant senescence processes. Additionally, Hong et al. (2005) reported that leaves of spinach (*Spinacia oleracea* L.) treated with TiO_2 nanoparticles had higher levels of photosynthesis compared to untreated leaves.

Several studies on the application of nanoparticles in a relatively broad range of species have attempted to understand the effect on plant growth. For instance, Lin and Xing (2007) reported that ZnO nanoparticles can inhibited seed germination of ryegrass. On the other hand, Stampoulis et al. (2009) did not found a cause-effect on seed germination, root elongation and biomass of zucchini (*Cucurbita pepo* L.) amended with nanoparticles in hydroponic solutions. In this study root fresh weight, root dry weight, root length, shoot fresh weight, shoot dry weight, shoot length, and SPAD units were not significantly different between nanoparticles treatments, compared to the CONTROL treatment ($P < 0.05$), i.e., NP did not change significantly some biomass parameters such as root or shoot dry weight. NP did not affect significantly the growing processes, but whether some characteristics related to yield components such as those linked to pods or seeds. It is assumed that in this study the NP-induced toxicity might not affect the plant growth. However, it has to be noted that other modes of actions as photo-induced toxicity and NP-dissolved ion effects might elicit toxicity (Fernández-Luqueño et al., 2014).

In this research, we found that at least one concentration of HEMATITE, FERRIHYDRITE or MAGNETITE increased significantly the total N of roots or shoots, the number of pods, dry weight of pods, the number of seeds, and yield of common bean. Burke et al. (2015) reported that Fe_3O_4 nanoparticles can affect the root system as wells as leaf phosphorous content from soybean plants (*Glycine max* (L.) Merr.), but Quoc et al. (2014) found that iron NP increased up 16% the yield of soybean in comparison with the control sample. In addition, Martinez-Fernandez et al. (2016) found reduction of the root functionality from sunflower plants (*Helianthus annuus* L.) by iron oxide nanoparticles. On the other hand, at least one concentration of HEMATITE, FERRIHYDRITE or MAGNETITE decreased significantly the fresh or dry weight of pods. Jeyasubramanian et al. (2016) stated that Fe_2O_3 nanoparticles increased the stem and root lengths and biomass production of spinach plant (*Spinacia oleracea* L.), while the effects were dependent of time and dose.

At least one concentration of ZINC OXIDE or TITANIUM DIOXIDE decreased significantly the number of pods, the fresh weight of pods and the number of seeds. Jacob et al. (2013) found that TiO_2 NP did not affect biomass production in common bean plants grown in nutrient solutions at 0, 6, and 18 mmol Ti L^{-1}. However, Adhikari et al. (2016) stated that application of nano-zinc oxide particles enhanced the auxin

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indole-3-acetic acid (IAA) production in plant roots of maize (*Zea mays* L.), soybean (*Glycine max* L.), pigeon pea (*Cajanas cajan* L.), and ladies finger (*Abelmoschus esculentus* L.), which subsequently improved the overall growth. In addition, it has been reported that independent of NP type, a concentration of 250 mg kg^{-1} of TiO_2 and ZnO NP promoted the highest plant height, root length, and biomass (Raliya et al., 2015). These authors stated that zinc oxide NP had a twin role of being an essential nutrient and a co-factor for nutrient mobilizing enzymes.

It is well known that NP are up taken by the vascular network but the accumulation rate in tissue is different for root and shoot systems from each plant species, while each NP type might have a differential interaction ship with cells as effect of the growing stage, NP size, time exposition, and biotic and abiotic factors. These considerations could be the main reason for the wide variability of results when attempting know the effects of NP on plants. Additionally, it has to be highlighted that some plants NP-treated do not show any observable phenotypic changes in overall growth indicating that environmental NP pollution could be dangerously unnoticed.

Conclusions

None of the five kinds of NP used in this experiment (magnetite, ferrihydrite, hematite, zinc oxide or titanium dioxide) modified significantly the chlorophyll content of common bean plants as witnessed by the SPAD units' values. However, nanoparticles of magnetite, ferrihydrite, hematite, zinc oxide or titanium dioxide modified significantly at least one plant characteristic or one yield component of common bean, such as SPAD units, root length, root total N, shoot length, shoot total nitrogen, pod number, pod fresh weight, pod dry weight, seed number or yield. The nanoparticles with Fe such as magnetite, ferrihydrite, or hematite were those that increased significantly more crops characteristics such as total N of roots or shoots, the number of pods, dry weight of pods, the number of seeds, and yield of common bean. These finds are an important factor to take into account with regard to the applicability of NP for long-term use in crops but, the selection of the proper NP at their adequate concentration is important for realizing higher benefits for an agrosustainable target. Additionally, there is the need of generating more data on chronic effects from long terms and concentration exposure of nanoparticles in plants, which is important for a better understanding of the potential hazard or risk of these nanoparticles, while more studies are also necessaries in order to identify the highest potential of NP in the rural sector and in the agro-food industry worldwide.

Acknowledgements. This research was founded by 'Ciencia Básica SEP-CONACyT' projects 151881 and 287225, the Sustainability of Natural Resources and Energy Program (Cinvestav-Saltillo), and Cinvestav Zacatenco. M-P G. received grant-aided support from 'Becas Conacyt'. F-L F, T-T LI, L-V F, and P-G L received grant-aided support from 'Sistema Nacional de Investigadores (SNI)', Mexico.

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DOI: http://dx.doi.org/10.15666/aeer/1602_18831897