INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1314/2018/20–8–1818–1824
DOI: 10.17957/IJAB/15.0698
http://www.fspublishers.org

Full Length Article

Effect of Phosphorus Rates and *Bacillus subtilis* on Growth, Dry Matter Production and Yield of Common Bean in Sinaloa, Mexico

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Abstract

Common bean (*Phaseolus vulgaris* L.) is an important legume that constitutes part of the daily feeding in countries like Mexico. In Northern Sinaloa, Mexico, bean yield remains low due to management and environmental factors that affect yield potential. Therefore, a field experiment was conducted in this region in order to investigate the response of common bean to different rates of phosphorus fertilizer and the role of *Bacillus subtilis* strain Q11 inoculation on growth, dry matter production and yield potential of the crop. The experiment was conducted as a split plot in a randomized complete block design with three replicates. A factor evaluated was four P rates (0, 25, 50, 100 kg ha^{-1}) and other was the inoculation or not inoculation with *B. subtilis*. Variables as height, canopy closure, growth index, NDVI, yield parameters, and yield were evaluated. Based on the results obtained, P x *B. subtilis* interaction was not significantly (P>0.05) for the majority of parameters evaluated. Only P rates significantly (P<0.05) enhanced all variables of growth as a function of time. Usually, rates of 50 or 100 kg P ha^{-1}, were sufficient to enhance the growth components of stems and roots and dry matter production. In conclusion it was found that rate of 50 kg ha^{-1} was the more viable in enhancing the maximum growth, dry matter production and yield of common bean cultivated under this environment and under this soil conditions. © 2018 Friends Science Publishers

Keywords: Common bean; Dry matter; Fertilizer rates; Nutrients; Soil bacterium

Introduction

Legume crops have become an important source for human nutrition worldwide after cereals (Anderson *et al*., 2004). In Mexico, common bean ranks the fourth position due to land surface established. Recently, one of the most concerning factors that cause declines in production in areas where the crop is planted are water deficit and improper fertilization/irrigation. Despite the fact that common bean has the ability to fix atmospheric nitrogen regardless of soil fertility and mineral nutrition, it requires high nitrogen inputs for good growth and development (Withers *et al.*, 2014). Actually, growers of northern Sinaloa apply high nitrogen rates to maximize yield of this crop due to low solubility and high costs of common P fertilizers.

The role of P as a major nutrient in plants it is well known, thus the importance of the behavior of P fertilizers in soils that can influence or affect growth and/or yield of a crop is also a crucial process in fertilization management programs.

The majority of P is present as low available forms due to reactions that occur in soils (Ryan *et al.*, 2012). One of them is precipitation in calcareous soils with high pH and low organic matter content (Amanullah and Khan, 2015). Therefore, some researchers suggest more efficient fertilization practices as well as the use of microorganisms to improve P availability, crop productivity and soil sustainability (Patel *et al.*, 2010; Simpson *et al.*, 2011; Wang *et al.*, 2011; Withers *et al.*, 2014; Heppell *et al.*, 2015).

The most studied plant growth promoting rhizobacteria genera are *Bacillus*, *Pseudomonas*, *Rhizobium* and *Enterobacter* (Araujo, 2008). However, their action could be affected by the sorption capacity of the growing medium and the solubility of P forms in it (Rodriguez *et al.*, 2006). In addition, their effects occur indirectly through reduction of plant pathogens agents (De Santiago *et al.*, 2011; Yuan *et al.*, 2012) and directly by production of organic acids and acid phosphatases which solubilize nutrients especially phosphates (Mena and Portugal, 2007; Khan *et al.*, 2013; Sarwar *et al.*, 2016).

Actually, the information concerning accurate rates of P as well as the use of the inoculant *Bacillus subtilis* Q11 as P solubilizer/growth promoter of common bean in northern

To cite this paper: Ruelas, J.D.R.I., Á.R. Olivas, F. Núñez-Ramírez, J.F. Velázquez and F.V. Guerrero, 2018. Effect of phosphorus rates and *Bacillus subtilis* on growth, dry matter production and yield of common bean in Sinaloa, Mexico. *Int. J. Agric. Biol.*, 20: 1818–1824

Sinaloa is scarce. So far the reports of this strain have shown to have an antagonistic effect against *Sclerotium rolfsii* and other soil borne pathogens (Hernandez *et al.*, 2016). Therefore, the aim of this research was to evaluate four P fertilizer rates combined or not with *Bacillus subtilis* Q11 on growth, dry matter production and yield of common bean.

Materials and Methods

The field experiment was conducted during the winter season at Valle del Fuerte located northern Sinaloa, Mexico (25° 45´49" N, 108° 51´41" W). The weather is hot during summer (maximum temperatures up to 40°C) and moderate cold during winter (12–30°C). The annual precipitation ranges between 700–750 mm and its distribution is highly variable.

Soil tilling techniques were realized following the guideline provided by the Forestry, Agriculture and Livestock National Research Institute (SIAP, 2013). Soil samples at 30 cm were collected before fertilizer application (Table 1).

Nitrogen (N) was applied at a rate of 150 kg ha^{-1}. Total N and P were applied preplant using highly soluble Blaukorn® Classic (12-8-16). The variety planted was Azufrado Higuera. The experiment was carried out as a split plot in a randomized complete block design with three replicates. The main plot consisted of four P rates (0, 25, 50 and 100 kg ha^{-1}) whereas the subplots consisted of the inoculation of *Bacillus subtilis* Q11 and the control. Each main plot had a dimension of 64 m^2 while the subplots were 32 m^2 (4 rows wide arranged linearly head to tail of the field) within the main plot.

The planting was done on moistened soil and pest management was successfully controlled throughout the season. Irrigation scheduling was managed by the water balance method with the use of IrriModel software (Sifuentes *et al.*, 2012) which estimates the depletion levels according to the methodology proposed by Ojeda *et al*. (2004). Irrigation targets were set as a 50% depletion of plant available water, monitoring water content with Time Domain Reflectometry.

Measurements

Plant growth measurements were done from five plants of the each treatment. Such parameters were plant height, canopy closure, growth index and normalized difference vegetation index (NDVI) readings. Plant measurements were made every other week representing the following stages of growth: third trifoliate leaf, flowering, pod filling, and physiological maturity.

Growth index was realized following the methodology proposed by Atland *et al*. (2003), Torres *et al.* (2017), which is the sum of the height, side width and front width of the plant, then divided by three. The scanning for NDVI was performed as an indicator of biomass production (indirect measure of crop growth) by using greenseeker handheld crop sensor (Trimble® Navigation Ltd., Sunnyvale, CA). The sensor was placed at approximately 50 cm above plant canopy on a row segment.

Additionally at pod filling stage, other crop growth parameters as root length, stem diameter, stem dry weight and, root fresh and dry weight were also measured. Fresh matter sampling consisted on harvesting 1.0 m of complete plants per plot. Plant material was separated in organs as roots, leaves, stems and pods, and weighed. After then, plant material was dried in an oven and newly weighed.

At the end of experiment, aboveground biomass yield and its components were determined from two central rows. Pod dry weight, percent of shelling, grain dry weight, dry weight of 100 grains, seed yield, dry matter of complete plants and harvest index were determined. All parameters were statistically analyzed (ANOVA) (Minitab, 2017).

Results

Plant Height, Canopy Closure and Growth Index

In this study, *B. subitilis* did not significantly affect (P>0.05) plant height, canopy closure and growth index parameters (Table 2); On the other hand, P rates applied revealed highly significant differences (P<0.05) on plant height as a function of time. The maximum heights values were achieved at 60 DAP (days after planting) with 54, 61, 65 and 68 cm for 0, 25, 50, and 100 kg ha^{-1} respectively. At the end of the season, the maximum heights were 31, 30, 41 and 45 cm for the above treatments.

Canopy closure was also significantly enhanced by P rates as a function of time (P<0.05). No differences (P>0.05) were found between treatments at 15 and 45 DAP. However, the crop had a rapid canopy closure on treatments that received 50 and 100 kg ha^{-1}, reaching its maximum canopy at 60 days after planting. As the crop reached maturity, it was also observed that plants on the same rates had delayed senescence as compared to the lower rates that had been mostly defoliated.

In regards the growth index pattern, the overall phosphorus rates significantly influenced this parameter as a function of time (P<0.05). It was observed an initial period of slow growth at third trifoliate leaf (15 DAP) increasing rates at flowering (30 DAP) reaching a plateau at pod filling stage (60 DAP) and then declining as the crop progressed to maturity (100 DAP).

The trend pattern showed that highest P rates exhibited the greatest growth as compared to the lowest rates (Table 2). According to data, all treatments had the following growth indexes at the end of season: 33, 32, 41 and 45, respectively, with no distinct growth pattern between the inoculated treatments and the control. In this study, it was found significant interaction effects (P<0.05) between P rates and the bacteria on plant height (30, 60 DAP), canopy closure (30, 75, 90, 105 DAP) and growth index

Table 1: Physical and chemical properties of the soil used for cultivation

Soil parameter	Values	Classification
Saturation (%)	50	low
pH (1:2 ratio)	7.6	alkaline
EC 25ºC (dS m^{-1})	0. 69	low
Nitrogen (mg kg^{-1})	145	High
Phosphorus (mg kg^{-1})	32	Medium
Potassium (mg kg^{-1})	760	High
Calcium (mg kg^{-1})	3950	High
Magnesium (mg kg^{-1})	590	High
Sodium (mg kg^{-1})	180	Low
Iron (mg kg^{-1})	6.40	Low
Manganese (mg kg^{-1})	11.96	High
Copper (mg kg^{-1})	1.82	Low
Zinc (mg kg^{-1})	1.48	Low

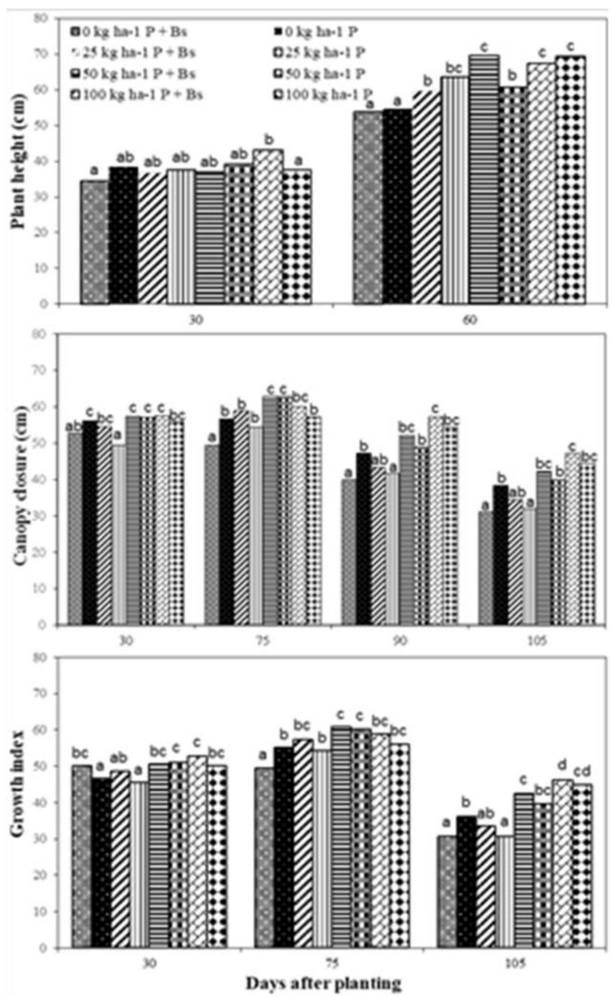

Fig. 1: Influence of phosphorus rates and *Bacillus subtilis* on parameters of growth as a function of time

(30, 75, 90 DAP; Fig. 1). in all cases from 30 DAP until 75 DAP, treatment 100 kg P ha^{-1} + *B. subtilis*, was outstanding over the 0 kg P ha^{-1} and for treatment 0 kg P ha^{-1} + *B. subtilis*. the time between 60 and 75 DAP was the most intense period of growth in common bean.

NDVI

As shown on Table 3, we have not found significant effect (P>0.05) on P x Mo interaction for NDVI parameter. Results shown only a significant response of P rates on NDVI values over the season, with no response of *B. subtilis.* Those values increased as early 45 DAP reaching a maximum at 60 DAP. At 75 DAP values started to low as the crop mature ending with values between 0.33 and 0.53. During the season, greatest values were significantly higher (P<0.05) for rates of 50 and 100 kg ha^{-1}.

Growth Components of Stems and Roots

Table 4 show the plant growth components measured at pod filling stage and its effect by P rates. Application of different P rates resulted in significant differences for almost all parameters except for root length (P>0.05). The overall stem diameter, root fresh and dry weight, as well as stem dry weight were in the order of 5.0 mm $plant^{-1}$, 15, 4.6 and 30 g $plant^{-1}$ approximately. In all cases, the rate of 50 kg ha^{-1} showed the highest values (P<0.05) whereas the rate of 100 kg ha^{-1} slightly decreased by 25, 18 and 13.5% for the last three parameters measured.

Yield and Yield Components

Table 5 show the response of pod dry weight, shelling percent, grain weight, weight of 100 grains, seed yield, dry matter of the biomass and harvest index by effect of four P rates. The application of 50 kg ha^{-1} was the optimal rate that significantly (P<0.05) increased pod dry weight, grain dry weight and 100 grain weight which were responsible of maximizing yield. The highest rate (100 kg ha^{-1}) decreased by 19, 5 and 7.5% over the medium rate (50 kg ha^{-1}) for each parameter. On the other hand, harvest index and shelling percentage were statistically affected neither by P rates nor for the application of *B. subtilis* (data no shown). The highest yield (3232 kg ha^{-1}) was with 50 kg P ha^{-1} but equal with 100 kg P ha^{-1}, followed by 25 kg P ha^{-1} and the control.

Discussion

The results showed that application of P rates by did have an influence on plant height and canopy closure. These results are in contrast to those reported by Turuko and Mohammed (2014) that found a non significant response of plant height to different P rates in a humid environment. They indicated that part of the P applied had been taken up by plants, adsorbed on mineral surfaces or precipitated as secondary P minerals.

On the other hand, treatments inoculated with *B. subtilis* did not significantly enhance (P>0.05) plant height or canopy closure compared to the control. A possible cause might be that some bacteria are preferred for certain

Table 2: Plant height, canopy closure and growth index as affected by phosphorus rates and *Bacillus subtilis* as a function of time intervals

Plant height	Days after planting						
Phosphorus rates (kg ha^{-1})	15	30	45	60	75	90	105
0	12.50 b	36.44 b	38.33 b	54.28 c	50.84 b	36.95 b	31.00 c
25	14.00 ab	37.27 ab	38.27 b	61.72 b	54.33 a	40.28 b	30.00 c
50	15.27 a	38.00 ab	42.55 a	65.17 ab	56.11 a	51.39 a	40.83 b
100	15.05 a	40.39 a	40.89 a	68.44 a	55.27 a	55.00 a	45.00 a
Probability	0.007	0.024	0.004	<0.0001	0.001	<0.0001	<0.0001
Microorganism (Mo)							
Bacillus subtilis	14.22	37.88 a	40.33	62.67 a	54.44	46.67	37.28
control	14.19	38.16 b	39.69	62.14 b	53.83	45.14	36.14
Probability	0.959	0.744	0.434	0.665	0.453	0.213	0.216
P*Mo Probability	0.340	0.005	0.902	0.009	0.097	0.254	0.055
Canopy closure							
Phosphorus rates (kg ha^{-1})							
0	21.11	54.39 bc	66.89	80.22 b	53.07 c	43.61 c	34.73 c
25	20.66	52.00 c	66.11	80.22 b	56.66 bc	42.50 c	33.22 c
50	21.77	57.27 a	66.11	84.28 a	62.778 a	50.56 b	41.11 b
100	21.05	56.72 ab	70.83	82.28 ab	58.60 b	55.83 a	45.83 a
Probability	0.372	0.001	0.334	0.0115	0.001	0.001	0.001
Microorganism (Mo)							
Bacillus subtilis	21.47	55.55	67.64	80.86	57.78	48.19	38.75
control	20.83	54.64	67.33	82.63	57.78	48.05	38.70
Probability	0.163	0.177	0.884	0.184	0.999	0.910	0.967
P*Mo Probability	0.121	0.002	0.205	0.988	0.001	0.024	0.049
Growth index							
Phosphorus rates (kg ha^{-1})							
0	18.24 b	48.40 b	57.37	74.28 b	52.31c	41.39 c	33.48 c
25	18.44 ab	47.09 b	56.83	74.06 b	55.889 b	41.76 c	32.15 c
50	19.61 a	50.85 a	58.26	75.20 ab	60.556 a	50.83 b	41.02 b
100	19.05 ab	51.27 a	60.852	77.66 a	57.500 b	55.55 a	45.56 a
Probability	0.049	0.001	0.269	0.041	0.001	0.001	0.001
Microorganism (Mo)							
Bacillus subtilis	19.04	49.66	58.54	74.79	56.67	47.69	38.26
control	18.63	49.14	58.12	75.80	56.46	47.08	37.84
Probability	0.296	0.372	0.783	0.343	0.773	0.597	0.711
P*Mo Probability	0.241	0.001	0.271	0.428	0.001	0.067	0.050

Means followed by different letters in a column are statistically different (Fisher $P \leq 0.05$)

Table 3: NDVI readings as affected by phpsphorus rates and *Bacillus subtilis* as a function of time intervals

NDVI	Days after planting						
Phosphorus rates (kg ha^{-1})	15	30	45	60	75	90	105
0	0.75	0.80 b	0.83 b	0.82 b	0.59 b	0.33 b	0.33 b
25	0.71	0.83 a	0.85 ab	0.84 a	0.62 b	0.36 b	0.36 b
50	0.73	0.85 a	0.86 a	0.85 a	0.69 a	0.47 a	0.47 a
100	0.73	0.84 a	0.85 a	0.84 a	0.73 a	0.53 a	0.53 a
Probability	0.540	0.002	0.017	0.029	0.040	0.001	0.001
Microorganism (Mo)							
Bacillus subtilis	0.74	0.83	0.85	0.84	0.65	0.43	0.43
control	0.72	0.83	0.85	0.83	0.66	0.42	0.42
Probability	0.328	0.350	0.895	0.415	0.846	0.668	0.668
P *M Probability	0.790	0.989	0.452	0.505	0.795	0.983	0.983

Means followed by different letters in a column are statistically different (Fisher $P \leq 0.05$)

crops (Bashan, 1998), some others are more or less efficient in competing with other microorganisms and solubilizing P (Khalid *et al*., 2004), while others are affected by soil conditions in the rhizosphere (Kannan *et al*., 2005; Lalfakzual *et al*., 2008).

Regarding the overall growth index, data showed that the most efficient rate was with 50 kg ha^{-1} over the course of the season. The slow response of the crop to the highest P rate (100 kg ha^{-1}) could have been possibly attributed to the following factors as mentioned by some authors: changes in soil solution pH after fertilizer application (Sanchez, 2007) or low efficiency of plant uptake related to NH_4^+ toxicity affecting plant growth (Havlin *et al.,* 2005).

In addition, it was found that *B. subtilis* did not exert a positive effect on growth index probably because N_2 fixation was limited as observed with poor root nodulation and that solubilization of other nutrients was relatively scarce to plants. These results are different from those

Table 4: Growth components of common bean at pod filling stage (60 DAP) by effect of four P rates

Phosphorus rate (kg ha^{-1})	Root length (cm)	Stem diameter (mm plant^{-1})	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})	Stem dry weight (g plant^{-1})
0	16.40	4.26 b	13.80 b	3.93 c	24.3 c
25	19.58	5.23 a	13.90 b	5.06 ab	27.3 c
50	21.50	5.05 a	19.26 a	5.33 a	37.0 a
100	20.50	5.51 a	14.35 b	4.38 bc	32.0 b
Probability	0.052	0.010	0.001	0.010	<0.001

Means followed by different letters in a column are statistically different (Fisher $P \leq 0.05$)

Table 5: Yield and its components of common bean by effect of four P rates

Phosphorus rates (kg ha^{-1})	Pod dry weight (g)	Shelling (%)	Grain dry weight (g)	Dry weight of 100 grains (g)	Seed yield (kg ha^{-1})	Dry matter biomass (kg ha^{-1})	Harvest index (%)
0	220.3 b	77	161.6 c	37.67 c	2861.8 b	10 989 c	49
25	231.2 b	74	168 bc	42.06 b	2786.2 b	12 989 b	58
50	289.0 a	79	195.4 a	46.58 a	3232.0 a	14 776 a	58
100	234.6 b	70	185.7 ab	43.02 ab	3087.0 ab	13 657 ab	55
Probability	0.020	0.065	0.010	0.001	0.040	0.001	0.061

Means followed by different letters in a column are statistically different (Fisher $P \leq 0.05$)

reported by Joe and Sivakumaar (2009) who found that inoculating with PGPR positively enhanced growth of sunflower crop. Others as Sarwar *et al.* (2016) reported a better performance of P fertilizers and *Pseudomonas i*solates rather than *Bacillus.*

On the other hand, the interaction of P X *B. subtilis* was evident during the period of 30 until 75 DDS. In this sense, Korir *et al*. (2017), found a high number of nodules during the same period of time. They also found the largest number of nodules in the inoculated plants unlike the non-inoculated plants.

Additionally, the highest NDVI values obtained on the highest P rates showed a strong relationship with dry matter accumulation at 75 DAP; in addition to yield at 45, 90 and 105 DAP which could be a direct effect mainly attributed to crop nutrient acquisition on stages of maximum demand; so that these NDVI values could further be used as an indirect measure of biological yield (Gutierrez *et al.*, 2016) and yield prediction.

The moderate rate (50 kg) performed better on those parameters probably because it did not cause any toxicity as a result of the rate applied. In contrast to the rate of 100 kg ha^{-1} that did not influence positively those parameters probably because adsorption reactions could have been stronger at the zone where fertilizer was applied (Havlin *et al.*, 2005), or because part of phosphorus could have been strongly retained in the area of contact (Sanchez, 2007).

This work differs from previous findings by Abbasi *et al.* (2008) and Zafar *et al.* (2011) who reported that plant dramatically enhanced shoot growth and leaf expansion with the highest P rate. Inoculation with *B. subtilis* showed no influence as compared with the control; which could also be related to its lower viability after inoculation. However, this result is different from previous findings which reported that application of PGPR significantly increased bean growth parameters (Remans *et al.*, 2007; Yadegari *et al.*, 2010; Abbasi *et al.*, 2011; Stajkovic *et al.*, 2011; Sharma *et al.*, 2013; Singh, 2013).

In this study, all P rates affected differently the pattern of dry matter accumulation. There was a variable response of the crop as observed directly on plant architecture. In that 50 kg ha^{-1} was the most efficient rate in terms of adequate supply of P that increased this parameter as compared to the highest rate (100 kg ha^{-1}). This results are similar from Meseret and Amin (2014) who achieved the greatest dry matter production with medium P rate. These results are different on those reporting that plant growth promoting rhizobacteria along with P fertilizers promoted the production of dry matter (Sharma and Prasad, 2003; Gupta *et al*., 2009; Joe and Sivakumaar, 2009).

Yield components were significantly affected by P rates (P<0.05). Application of 50 kg ha^{-1} showed to be the optimal rate that significantly increased pod dry weight, grain dry weight and 100 grain weight which in turn, were responsible of maximizing yield. This result is in agreement with those researchers who found the highest yield with the medium rate. Besides, the lowest rates (25 kg ha^{-1} P and 0 kg ha^{-1}) showed to have limited concentrations in soil that were not enough to positively influence parameters of yield potential. The above was probably as a result of nutrient dilution in the plant by its own growth.

Finally, harvest index and shelling percentage were not statistically affected by P rates or by the inoculation with *B. subtilis*. The efficiency of the inoculation with *B. subtilis* did not have any effect on these parameter evaluated because did not reach to increase sufficient reproductive organs that modify appreciably this factor. That was in unlike with some other studies as those realized by Selvakumari *et al*. (2000) and Roesty *et al*. (2006).

Conclusion

The results obtained in this study provide a nutrient management practice that could be applied in fields and subjected to further evaluation. The purpose is to have a basis on the best rate of phosphorus fertilizer rate as well as to identify the most active stages where growth, dry matter accumulation and yield can be maximized. The rate of 50 kg ha^{-1} positively increased the yield potential of the crop. However, more research is needed on the same rates in different soils as well as the sole growth promoting effects, P solubilizing capacity of *B. subtilis* Q11 and soil interactions that hinders the uptake by plants.

Acknowledgements

The first author acknowledges the financial grant from Química International Aplicada S.A de C.V, and the student scholarship from Consejo Nacional de Ciencia y Tecnología, México.

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(Received 20 November2017; Accepted 06 April 2018)