Middle East Journal of Agriculture Research
ISSN 2077-4605

Volume : 04 | Issue : 04 | Oct.-Dec. |2015
Pages: 932-937

Effect of Some Microbial Activators on Onion Plantlets Growth Quality

Khadiga I. M. El-Gabry, Sanaa S.H. Sarabana and Amal W. Abou El- Khair

Soils, Water and Environ. Res. Inst., Agric. Res. Center, (ARC), Giza, Egypt.

ABSTRACT

A biotechnological procedure in agriculture is based on the activity and biomass of rhizospheric microbial for plant productivity and soil quality. Three strains of effective Plant growth promoting rhizobacteria (PGPR) (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae*) were selected to have maximum benefits from this technology. They were used as single and mixed inoculants for studying their effect on onion plantlets growth quality grown in greenhouse pot experiment in Microbiology Department, Soils, Water and Environment Res. Inst., Agric. Res. Cent., Giza. Results showed statistically significant differences between the treatments inoculated with the three PGPRs and the control. Microbial activities (in terms of CO_2 and dehydrogenase activity) in rhizosphere and nutrient content in onion (nitrogen, phosphorus and potassium) were investigated after 70 days of sowing. The highest dehydrogenase activity and CO_2 was obtained due to consortium inoculation with the three PGPRs strains (33.53 µg triphenyl formazan g^{-1} soil $24hr^{-1}$ and 203.31 mg $100g^{-1}$ soil, respectively). It also demonstrated an increase in the plant height (68.91 cm). Also, there was a significant increase in nitrogen and phosphorus content in the treatment of *Ps.* Mixed with yeast (5.07 and 0.37%, respectively) while the mixture of *Bacillus* with yeast demonstrated the highest potassium content (2.24%). Plant analysis (total soluble solids, number of leaves/plant, single bulb, bulb weight and double bulbs) was done after 130 days at the harvest. Investigations indicated that soil inoculated with the three selected PGPR strains is required for maximizing onion yield as single bulbs (86.4%) with weight of 72.41 g and a decreasing in the double bulbs (2.1%).

Key words: *Bacillus subtilis, Pseudomonas fluorescens,* yeast*, Saccharomyces cerevisiae,* Plant growth promoting rhizobacteria (PGPR), Onion.

Introduction

PGPR can be applied in agricultural production or for the phyto-remediation of pollutants. However, because of their capacity to confer plant beneficial effects, efficient colonization of the plant tissues is almost important. Additionally, more than 50% of the applied inorganic N-fertilizer is always lost through different processes e.g., denitrification and consequently may make environment pollution. The majority of plant-associated bacteria derived from the soil environment may migrate to the rhizosphere of their hosts before they are able to show beneficial effects.

The understanding of colonization process is important to better prediction of how bacteria interact with plants and they are likely to establish themselves in the plant environment after planting as result of their application as biofertilizers or biocontrol agents (Stephane *et al*., 2010). Hartmann *et al.,* (2008) reported that the soil environment attached to the root system is a hot spot of microbial abundance and their activity may due to the presence of root exudates and rhizo-deposits.

Some rhizosphere microorganisms may be neutral or deleterious with regard to plant growth, whereas other microbes support their hosts (Raaijmakers *et al.,* 2008). Such PGPR can stimulate plant growth, increase yield, reduce pathogen infection, as well as reduce biotic or abiotic plant stress without conferring pathogenicity (Lugtenberg and Kamilova, 2009).

Excessive use of organo-stimulant may exert deleterious effects on microorganisms, which adversely affect fertility as well as pollute the environment. Use of organo-stimulant of control of pathogens is effective and convenient but their use and abuse are causing serious ecological economic and social problems. Alternative approaches are needed to substitute the organo-stimulants with bacterial fertilizers and bio-pesticides because of the potential threat for development of organo-stimulant resistance especially systemic fungicides, by fungal pathogens and non-target side effects on other plant pathogens and beneficial microorganisms (Harish *et al.,* 2010).

Interactions between indol acetic acid (IAA)-producing bacteria and plants lead to diverse outcomes on the plant side, varying from pathogenesis to phyto-stimulation. Reviewing the role of bacterial IAA in different

Corresponding Author: Khadiga I.M. El-Gabry, Soils, Water and Environ. Res. Inst., Agric. Res. Center, (ARC), Giza, Egypt.
E-mail: Khadigagabry@yahoo.com

microorganism–plant interactions highlights the fact that bacteria use this phyto-hormone to interact with plants as part of their colonization strategy, including phyto-stimulation and circumvention of basal plant defense mechanisms. (Stijn *et al.,* 2007).

The level of expression of IAA depends on the biosynthesis pathway; the location of the genes involved, either on chromosomal or plasmid DNA, and their regulatory sequences; and the presence of enzymes that can convert active free IAA into an inactive conjugated form. The role of bacterial IAA in the stimulation of plant growth and phyto-pathogenesis is considered (Cheryl and Bernard, 1996). *B. subtilis*, *Ps. fluorescens* and yeast were used by Udagwa and Kinoshita (1961) and Glickman and Dessaux, (1995) as active producers of gibberellins (GA) and indol acetic acid (IAA). Particularly the first one produced 192 and 851 mg l^{-1} of IAA and GA, respectively. While the second recorded 163 and 509 mg l^{-1} of IAA and GA, respectively.

Therefore, the aim of this work was that how to benefit from the bio-fertilizers to give higher yield of single bulbs of onion with a decreasing in the apparition of double bulbs (high onion quality).

Material and Methods

Microbial inoculate:

Rhizobacteria were used either separately or in mixtures for inoculating plantlets of onion (C.V. Giza 6). Combination of microbial inoculants was prepared by mixing equal aliquots of log phase (10^7 cells ml^{-1}) of cultures grown on King's Medium (peptone, 20g/l; $MgSO_4.H_2O$, 1.5g/l; $K_3PO_4.3H_2O$, 1.8 g/l; glycerol, 10 g/l). Plantlets were socked in microbial inoculate for 3 hours then it was sown. Foliar spray of the three strains was applied at three time intervals 21, 42 and 52 days from sowing.

The treatments were allocated as follows:

1- Control without inoculation.
2- Inoculation with *Bacillus subtilis.*
3- Inoculation with *Pseudomonas fluorescens.*
4- Inoculation with *B. subtilis* + Yeast.
5- Inoculation with *Ps. fluorescens* + Yeast.
6- Inoculation with *B. subtilis* + *Ps. fluorescens* + Yeast.

All treatments received the recommended dose of ammonium sulfate (100 kg fed^{-1}), Superphosphate (200 kg fed^{-1}) and Potassium sulfate (50 kg fed^{-1}).

Table 1: Physio-chemical properties of the experimental soil.

Soil characteristics	Values
Coarse sand (%)	13.8
Fine sand (%)	24.3
Silt (%)	29.1
Clay (%)	32.8
Texture clay	Clay
$CaCO_3$ (%)	1.36
pH (1:25) soil solution	7.1
EC dS m^{-1} (soil paste)	1.84
Total N (%)	0.04
Organic C (%)	0.78
Cations (mg L^{-1})	
Ca^{++}	7.02
Mg^{++}	7.89
Na^{+}	10.37
K^{+}	0.63
Anions (mg L^{-1})	
CO_3^{-2}	0
HCO_3^{-1}	2.29
Cl^{-}	9.04
So_4^{-2}	14.58

Pots experiment:

Pots experiment was execute under greenhouse conditions (20 cm in diameter) in Microbiology Department, Soils, Water and Environment Res. Inst., Agric. Res. Cent., Giza. Every pot was filled with 2 kg soil, soil analysis was shown in table (1). Plantlets of onion (C.V. Giza 6) were washed with tap water and socked in the microbial inoculants. Samples were taken after 70 days of sowing to measured plant height fresh and dry weights (drying at 70^{o} C over night) as well as determining nutrient contents (N.P. and K.) according to Page *et al.*, (1982), dehydrogenase activity according to Thalmann, (1967), soil biological activity in terms of CO_2 as described by Gaur *et al.*, (1971) and count of bacteria and yeast according to Allen, (1959). At harvest, onion plants (Giza 6) were collected after 130 days for determining total soluble solids (TSS), Number of leaves/Plant (No L P^{-1}), single bulb (SB), bulb weight (B Wt) and double bulbs (DB). The obtained results were statistically analysis according to Gomez and Gomez, (1984).

Results and Discussion

A biotechnological procedure in agriculture is based on the activity and biomass of rhizospheric microbial for plant productivity and soil quality. Selection of effective PGPR is the most critical aspect to have maximum benefits from this technology (Hosam *et al.,* 2013). The PGPR are known to promote plant growth through several mechanisms. Indirect mechanisms involve improving nutrient availability or preventing growth of pathogenic microorganisms, while direct mechanisms include either altering plant hormonal balance or inducing the plant defensive systemic response (Ramos *et al.,* 2008). Hosam *et al.,* (2013) mentioned that soil inoculation by plant growth promoting rhizomicrobiota (PGPR) especially species of soil useful microbiota e.g., *Pseudomonas, Rhizobium, Bacillus, Trichoderma* and *Saccharomyces* are model microorganisms to demonstrate influence on plant health.

In this search, three strains of PGPR (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae*) were used as single or mixed inoculants and were foliated on the plantlets of onion (Giza 6) in greenhouse pot experiments at three time intervals (21, 42 and 52 days from sowing) to study their effect on it.

Microbial activities in the rhizosphere of onion:

Data in Table (2) showed that there were more significant increases in CO_2 production in all tested treatment than the control. Maximum value (203.31mg/100g soil) was attended in rhizosphere onion for soil treated with mixture of *B. subtilis, Ps. fluorescens* and yeast. It was followed by the treatment of *Ps. fluorescens* alone which produced 145.170 mg/100 g soil. This result indicated that the strain of *Ps. fluorescens* was the more active PGPR in its high energy of metabolism reflecting the high productivity of CO_2 in the rhizosphere. The production of CO_2 was led to in formation of carbonic acid that decreases the soil pH value at rhizosphere. This process had done by rhizosphere microorganism's which act to increase nutrient uptake and availability of the nutrient in the rhizosphere plant area which in turn supported higher plant growth and yield. These results were in agreement with Seied *et al.,* (2013) who indicated that the high CO_2 production rates reflect the high energy of metabolism.

Table 2: Effect of bio-fertilizers on the rhizosphere of onion and plant height.

Treatments	CO_2 (mg 100 g^{-1} soil)	De-Hase (µg triphenyl formazan g^{-1} soil $24hr^{-1}$)	Total Count (10^{-7} cfu g^{-1}soil)	Plant height (cm)
Control	76.767 f	15.23 d	8.313 d	60.40 f
B. subtilis	112.22 e	17.57 c	15.510 c	61.43 e
Ps. fluorescens	145.17 b	24.507 b	17.350 bc	62.32 d
B. subtilis +Yeast	133.42 d	26.63 b	19.510 b	64.15 c
Ps. fluorescens +Yeast	140.11 c	26.42 b	22.420 a	65.11 b
B. subtilis + *Ps. fluorescens* +Yeast	203.31 a	33.53 a	24.350 a	68.91 a
LSD at %5	1.903	2.124	2.285	0.834

The highest dehydrogenase activity (33.53 µg triphenyl formazan/g soil/24hr.) was also recorded with the bacterial and yeast mixture, followed by the other treatments containing *Ps. fluorescens* alone or mixed with yeast. But it was a significant difference between this result and that of the application of *B. subtilis* alone and also that of the control which were 17.570 and 15.230 µg triphenyl formazan g^{-1} soil $24hr^{-1}$, respectively. This means that the strain of *Ps. fluorescens* and the yeast strain were more active for the dehydrogenase activity than the Bacilli strain. Inoculation with PGPR significantly enhanced the activity and the dense population of soil microorganisms in the rhizosphere. So that increases their dehydrogenase activity. Dehydrogenase releases hydrogen ions in the rhizosphere resulting in formation of carbonic acid that decreases the pH value. Also this process had done by rhizosphere microorganisms which increase nutrient uptake and availability of the nutrient in the rhizosphere plant area. These results were in agreement with Omar and Ismail, (2002).

About the total count of microbes, it was also seen that the mixing strains were the best treatment (24.35 x 10^{-7} cfu g^{-1} soil) followed by Ps. *fluorescens* mixed with yeast (22.42 x 10^{-7} cfu g^{-1} soil). Also, the plant height was the best in case of addition of the two bacterial strains with yeast (68.91 cm). The results further prove previous established studies that state increase in the number of yeast cells in soil causes a significant rise in the growth of the plant in that soil. There are studies showing that yeast is capable of interaction with other symbiotic bacteria in natural conditions. This interaction may cause a more efficient nitrogen fixation amongst *Rhizobial* sp. Yeast such as *Saccharomyces* sp. not only affects plant and microbial growth but also plays a role in soil aggregate formation and maintaining structure of soil. This could be another reason as to the increased growth seen in treated plants (Chaitanya *et al.,* 2014).

Effect of bio-fertilizers on Macronutrients content in onion:

Data in Table (3) showed that all tested treatments increased significantly the nitrogen content in onion than the control, especially in treatment with the closed *Ps. fluorescens* and yeast (5.07 %), followed by that in closed *Bacillus* with yeast then the three mixed strains (4.240 and 4.147%, respectively). This result means that the application of yeast is important for increasing nitrogen content in onion. These findings are in an agreement with Hosam *et al.,* (2013) who mentioned that soil inoculation by effective combination of *Ps. fluorescens*, *B. subtilis* and *S. cerevisiae* as PGPR strains are required for maximizing plant yield and improve soil quality by increasing the organic carbon, total nitrogen and total phosphorus. Apart from this a large number of yeast sp. are also responsible for mineralization of organic materials, carbon and energy dissipation through soil. Major number of yeast species has also been linked to play some role in the natural nitrogen and sulphur cycle of soil (Chaitanya *et al.,* 2014).

The similar results were observed in phosphorus content which was 0.37 % in the treatment in closed *Ps. fluorescens* with yeast followed by the mixing all studied strains (0.31 %). Chaitanya *et al.,* (2014) mentioned that certain yeast sp. can solubilize insoluble inorganic phosphates and make them readily available for plants. Also, yeast strain could produce polyamines (spermine and spermidine) which increase cell division in roots leading to faster growth. Hosam *et al*., (2013) mentioned that inoculation with isolates of *Pseudomonas* had a stimulatory effect on plant growth and that it was between several of bacteria which could produce indolic compounds and siderophores, to solubilize phosphate.

Concerning the potassium content it attained was the higher value one when the onion was treated by *B. subtilis* mixed with yeast strain (2.24 %).

Generally, the increase in the macronutrient content may be due to the supply of Phosphorus that considered important for PGPR microorganisms which act to increase plant growth and Nitrogen translocation from root to shoots. Phosphorus plays a vital role in physiological and developmental process in plant life and it has favorable effect on accelerated the growth process through increase N uptake in plants. Similar results were shown by Zarrin *et al.,* (2007). Improved plant nutrition with PGPR might be due to induced systemic tolerance (IST) for example, if increased nutrient content in plants resulted from enhanced nutrient uptake, IST is operable because from physical or chemical changes in plant caused by PGPR which are ultimately responsible, as occurs when PGPR stimulate root development (Kloepper, 2004).

Table 3: Effect of bio-fertilizers on Macronutrients content in onion (after 70 days of planting).

Treatments	N (%)	P (%)	K (%)
Control	3.203 d	0.180 d	1.770 c
B. subtilis	2.977 d	0.170 d	1.650 D
Ps. fluorescens	3.760 c	0.200 cd	1.430 e
B. subtilis +Yeast	4.240 b	0.220 c	2.240 a
Ps. Fluorescens +Yeast	4.38 a	0.370 a	1.860 b
B. subtilis + *Ps. fluorescens* +Yeast	4.147 b	0.310 b	1.900 b
LSD at %5	0.330	0.034	0.043

Effect of bio-fertilizers on the onion quality:

Fig (1) showed that application of bio-fertilizers have a beneficial effect on the plant growth and yield quality. The treatment of composite of biofertilizers attended a significant value of yield quality parameters as compared with the all other treatments. So, it increased the total soluble solid (11.8%), the single bulbs (86.4%), the number of leaves (8.11 L P^{-1}) and bulb weight (72.41 g), while, it decreases the apparition of the double bulbs (2.1%). This could be attributed to the plant growth promoting substances produced by the bio-fertilizer. PGPR that exert beneficial effects on plant development often related to the increment of nutrient availability to host plant (Lug tenberg and Kamilova, 2009). Chaitanya *et al.,* (2014) explained that the growth promoting activity was due to the production of indole-3- acetic acid (IAA) and indole-3- pyruvic acid (IPYA) by endophytic yeasts. They observed significant increase in the growth of the plant by measuring root and shoot length, dry weight and also the in-planta levels of IAA and IPYA. Hosam *et al.,* (2013) showed that activities such as IAA like products, as well as P solubilization were among the most important activities of *Pseudomonas* sp.

The activity of PGPR colonizes plant roots leading to increase plant growth and yield due to their production of sidrophores and synthesis of antibiotics and enzymes has an important effect on production of phyto-hormones, on the availability and uptake of nutrients and depression of phyto-pathogenic microorganisms (Tilak *et al.,* 2006 and Mantelin and Touraine, 2004).

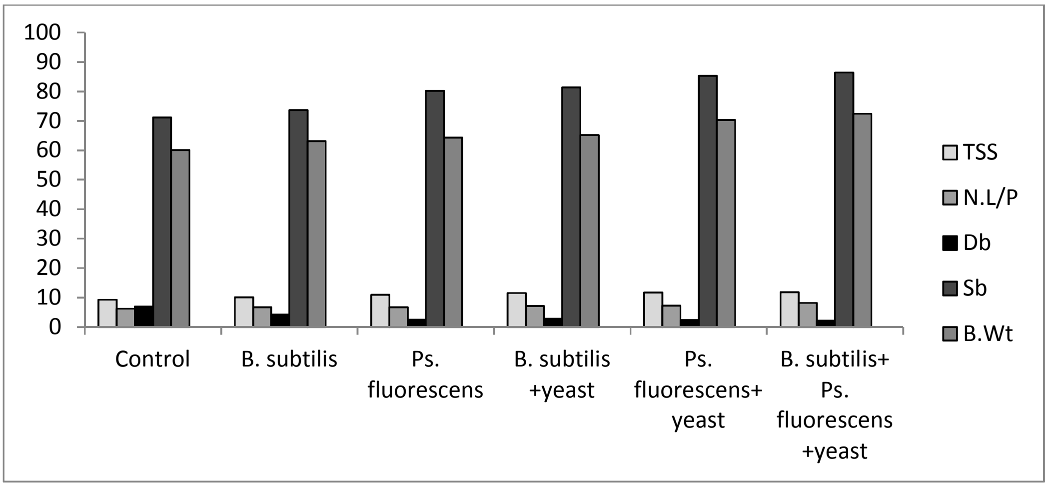

Fig. 1: Effect of bio-fertilizers on quality of onion harvest (130 days of sowing)
TSS: Total Soluble Solids, DB: Double Bulbs, SB: Single Bulb, No L P^{-1}: No. Leaves/Plant, B Wt: Bulb Weight

Conclusion:

The present study leads to conclude that the use of bio-fertilizers can benefit the onion production to give higher yield of single bulbs with a decreasing in the apparition of double bulbs and that it was important to supply yeast with the bacterial strains as effective PGPR. These results indicate a significant increment in the productivity of plants treated with these biofertilizers. It can be produced at a higher commercial level and its effect checked on various high yielding agricultural plants. So, it is necessary to continue researching in this field as it has the potential to be highly profitable for farmers as well as provide a way to a more sustainable future.

References

Allen, O.M., 1959. Experiments in soil bacteriology. (1st Ed.). Burgess Publishing Co., Minneapolis, Minnesota, U.S.A. Analysis of Experiments. Arnold a member of the Holder Headline Group. 1st Ed. London. UK. Biometrics., 11: 1-42.

Chaitanya, V., S. Mrudula, G. Tanvi, W. Samadhan and K. Chandrashekhar, 2014. Effect of *Rhizobium* Based Biofertilizer Combined with *Saccharomyces cerevisiae* on the Growth of Hyacinth Bean. International J. of Plant & Soil Science, 3(8): 959-968.

Cheryl, L. Patten and Bernard R. Glick, 1996. Bacterial biosynthesis of indole-3-acetic acid Canadian Journal of Microbiology, 42(3): 207-220.

Gaur, A.C., K.V. Sadasivan, O.P. Vimal and R.S. Mathur, 1971. A study of decomposition of organic matter in an alluvial soil. CO2 evolution microbiological and chemical transformation. Plant and Soil., 34: 17-28.

Glickmann, E. and Y. Dessaux, 1995. A Critical Examination of the Specificity of the salkowski reagent for indolic compounds produced by phytopathogenic Bacteria. Appl. Environ. Microbiol., February, 61(2): 793-796.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures of Agricultural Research. Second Ed. Wielly inter Sci. Publ. John Wiley and Sons. New York, pp: 357-423.

Harish, K., K.B. Vivek, R.C. Dubey, D.K. Maheshwari and C.K. Sun, 2010. Wilt disease management and enhancement of growth and yield of *Cajanus cajan* L *var.* Manak by bacterial combinations amended with organo-stimulant fertilizer. Crop Protection., 29: 591-598.

Hartmann, A., M. Rothballe and M. Schmid, 2008. Lorenz Hiltner a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant and Soil., 312: 7-14.

Hosam, E.A.F., Bayoumi Hamuda and István Patkó, 2013. PGPR strains selection to improve plant productivity and soil quality agricultural. Academy Food Research & Development Institute. International Scientific-Practical Conference. "Food, Technologies & Health", 2013. Proceedings Book.

Kloepper, J.W., 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* sp. Phytopathology, 94: 1259-1266.

Lugtenberg, B. and Kamilova, F., 2009. Plant Growth Promoting Rhizobacteria. Annual Review of Microbiol., (63): 541-556.

Mantelin, S. and B. Touraine, 2004. Plant Growth Promoting Bacteria and nitrate availability impacts on root development and nitrate uptake. J. Exp. Bot., 55: 27-34.

Omar, M.N.A. and H. Ismail, 2002. Complementation of diazotrophs and yeast as plant growth promoting agents for wheat plants. Egypt. J. Agric. Res., 80: 29-40.

Page, C.A., 1982. Methods of soil analysis. Amer. Soc. Agron. Madison, Wisconsin, U.S.A. soil CO_2 evolution, microbiological and chemical transformation. Plant and Soil., 34: 17-28.

Raaijmakers, J.M., T.C. Paulitz, C. Steinberg, C. Alabouvette and Y. Moenne-Loccoz, 2008. The rhizosphere: a playground and battlefield for soil borne pathogens and beneficial microorganisms. Plant and Soil., 321: 341-361.

Ramos, S.B., M. Barriuso and M.F. Gutierrez, 2008. Physiological and molecular mechanism of PGPR. Ch 3 in book: Plant-Bacteria Interactions: Strategies and Techniques to Promote Plant Growth, pp: 41-54.

Seied, N.E., R. Yaegoob, B.P. Amin and S. Bahzad, 2013. Effect of chemical fertilizers and bio-fertilizers application on some morpho-physiological characteristics of forage sorghum. Intr. J. Agron. And Plant Production., 4(2): 223-231.

Stephane, C., C. Christophe and S. Angela, 2010. Plant Growth Promoting Bacteria in the rhizo-and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. & Biochem., 42: 669-678.

Stijn Spaepen, Jos Vanderleyden and Roseline Remans, 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling, Microbiology Reviews, 31(14): 425-448.

Thalmann, A., 1967. Puber die microbiello aktivita undihr beziekung zu fruchtbartkeits merkmolen einiger Acherboden besouderer beruckrichtigung der dehydrogenas aktivitat. (TTC. Reduction). Biss. Gieben. Ph. D. Thesis, W. Germany.

Tilak, K.V.B.R., N. Ranganayaki and C. Manoharachari, 2006. Synergistic effects of Plant Growth Promoting Rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeon pea (*cajanus cajan).* European J. of Soil Sci., (57): 67-71.

Udagwa, K. and S. Kinoshita, 1961. A colorimetric determination of Gibberellic acid. J. Agric. Chem. Soc. Japan, 35: 219-223.

Zarrin, F., Z. Muhammad and C. Fayyaz, 2007. Interactive effect of Rhizobium strains and P on soybean yield, nitrogen fixation and soil fertility. Pak. J. Bot., 39(1): 255-264.