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Integrated Effects of Bio and Mineral Fertilizers and Humic Substances on Growth, Yield and Nutrient Contents of Fertigated Cowpea (*Vigna unguiculata* L.) Grown on Sandy Soils

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Abstract: A bio-fertigation trial was conducted at the experimental farm of National Research Centre, Noubaria, Egypt during May to August of 2010 to study the relative efficacy of bio-fertigation of liquid formulation of N-fixer (*Azospirillum* sp. and *Azotobacter* sp.) and P-solubilizer (*Basillus megatherium*) and humic substances (HS) and inorganic fertilizers injected through drip irrigation system on soil properties and growth and yield of cowpea plant. Four treatments were utilized mainly, basal control treatment at 100% recommended dose of NPK as drip fertigation while 50% recommended dose of NPK as drip fertigation combined with bio fertigation of microbial inoculums and/or Humic Substances (HS) were injected through drip irrigation system. Application of 50% recommended dose of NPK with bio fertigation and humic substances improved nutrient contents in soil (N, P, K, Fe, Mn and Zn), plant growth, nodule parameters, seed quality and fertilizer use efficiency and nutritional assimilation of cowpea followed by 100% recommended dose of NPK, 50% recommended dose of NPK jointly with humic substances and then 50% recommended dose of NPK in combination with bio fertigation treatments. In spite of 100% recommended NPK drip fertigation improved cowpea yield solely, application of 50% recommended NPK dose in conjunction with bio fertigation and humic substances gave rather equal yield. Injection of 50% recommended dose NPK as drip fertigation in combination with biofertigation of liquid formulation and humic substances was better and comparable way than the other treatments. Bio fertigation of microbial inoculums and humic substances could be used as a complementary for mineral fertilizers to improve yield and quality of cowpea under sandy soil conditions which protect the environment chemical pollution and its harmful effect on human and animal health.

Key words: Biofertigation, humic substances, *Vigna unguiculata* cowpea

INTRODUCTION

As the universe is going now on the way of clean agriculture and minimizing pollution effects, organic and biofertilisers became of the best management products to improve soil properties and productivity. They are considered as the most important factor in reducing the application of the inorganic fertilizers, consequently, reduce the adverse environmental impact of chemicals (Marschner, 1995). So, bio inoculants constitute an important component in integrated plant nutrient systems. It is an added advantage when these microbial inoculants are supplied through biofertigation as it has more water use efficiency and fertilizer use efficiency, quality etc. Effective microorganisms can also be applied in the filed along with organic or inorganic materials (Hussain *et al*., 1999).

The bio-fertigation can precisely deliver the bio-inoculants in the root zone. However, this technology needs to be standardized as the literature availability related to bio-fertigation is very scarce. *Rhizobium* spp. had significantly increased plant growth, nodulation and yield attributes in groundnut compared with individual inoculation (Madhaiyan *et al*., 2004, 2006; Aseri *et al*., 2008).

The organic and biological sources of nutrients provide essential nutrients to the crop and also enhance the positive interaction with chemical fertilizers by increasing their efficiency (Ahmed *et al*., 1996). Humic Substances (HS) are the most abundant organic constituents present in soil and aquatic environments those result from a humification process that involves microbial and chemical transformation of organic debris (Das and Ram, 2006). These stimulate microbial activity by providing the indigenous microbes with a carbon source for food, thus encouraging their growth and activity (Adani *et al*., 1998). Moreover, Taha *et al*. (2006) and Asik *et al*. (2009) concluded that humic substances

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gave the highest values of available nutrients, yield and nutrient uptake by wheat plant in different sandy soils. Lastly, Bama and Selvakumari (2001) found that application of 10 kg humic acid ha^{-1} as potassium humate along with 75% of recommended dose of nitrogen fertilizer increased crude protein content and mineral nutrition (P, K, Ca, Mg, Zn, Cu, Fe and Mn) of amaranths.

Cowpea (*Vigna unguiculata* L.) is a key staple food in many developing countries, and forms an integral part of the diet of about 120 million people around the world (IITA, 2004). It is a naturally N_2-fixing legume but it needs artificial inoculation with appropriate strains of rhizobia when introduced to land not previously cultivated to the crop (Ngakou *et al*., 2007). Under these conditions the poor growth and yield of cowpea plants could be ascribed to a low effectiveness of indigenous bradyrhizobial populations and to the low nutrients content in the soils. Therefore, the present study was undertaken to evaluate the performance of bio-fertigation under field condition and fertigation of inorganic fertilizers and humic substances on nodulation, seed yield and nutrients content of cowpea plants and soil properties.

MATERIALS AND METHODS

A bio fertigation trial was carried out during the (May-August) of 2006 summer season at the Experimental farm of National Research Centre, Noubaria, Egypt (30.73 N, 30.55 E). The soil is sandy in texture which comprising 86.7% sand, 8.3% silt and 5.0% clay. It is very poor in fertility; 0.85% OM with status of 26, 2.5, 95 mg kg $soil^{-1}$ of alkaline $KMnO_4$-N, Olsen s' P and NH_4OA_C-K, respectively and 3.13; 1.90; 0.85 of DTPA-extractable-Fe, Mn and Zn mg kg $soil^{-1}$, respectively. The pH is 7.94 and EC_e is 2.63 dS m^{-1}. The drip irrigation lines were GR (builtin-driper) lateral lines and the spacing between lateral lines was 50 cm with emitter spacing 16 mm outer diameter. The drippers used were of a standard 4L h^{-1} discharge at 1.5 bar working pressure. Irrigation water is very good with pH 7.4, EC 0.42 dS m^{-1} and 2.8 SAR. Bioinoculants were consisted of N-fixer (*Azospirillum* sp. and *Azotobacter* sp.) and P-solubilizer (*Basillus megatherium*). The efficient strains of bacteria in peat growth media were obtained from general organization for Agriculture Equalization Fund (GDAEF), Ministry of Agriculture and land reclamation, Egypt. Cultures of previous bioinoculatns at 500 mL (10^{12} cells mL^{-1}) was diluted in 500 L of water ha^{-1} and given as bio fertigation. Those were prepared in a container and allowed through the irrigation system at an interval of 7 days commencing from 15 days after sowing (DAS) up to 45 DAS (4 times). Bioinoculatns application through drip irrigation system was done at the second day after fertigation. Humic substances were applied at rate of 100 kg ha^{-1} produced from Egyptian Fertilizer Development Centre, Egypt. Humic substances had characteristics of pH 7.83; EC 0.94 and OM 68%. N, P and K were 3.40, 0.15 and 3.42%, respectively. Zn, Fe, Mn were 258, 415 and 214 mg kg DW^{-1}, respectively. Humic substances were divided into 4 equal doses and added through drip irrigation system at the same time of bio-fertigation.

Seeds of cowpea (*Vigna unguiculata* L.) cultivar Karim 7 were sown on 10 May 2006 and lasted for 100 days with spacing of 70 cm between the rows and 25 cm between the plants in a row. The experiment was laid out in a randomized block design and replicated three times in 5.4×4.8 m plot area.

The treatments used were namely, (1) drip fertigation with 100% recommended dose of NPK as control (T_1); (2) drip fertigation with 50% recommended dose of NPK plus humic substances (T_2); (3) drip fertigation with 50% recommended dose of NPK plus biofertigation (T_3) and (4) drip fertigation with 50% recommended dose of NPK plus humic substances plus biofertigation (T_4). Fertilizer in the compound form of 19N-19P-19K was added at the rates 100 and 50 kg ha^{-1} according to the treatments of NPK 100%; NPK 50% +HS; NPK 50% +BFM and NPK 50%+HS+BFM injected through drip irrigation system.

Leaf chlorophyll contents were measured by a Minolta SPAD-520 chlorophyll meter and readings were taken on 3rd leaf from. To analyze macro and micronutrients in cowpea organs (seed and straw), samples were taken from each plot and dried at 70°C and grounded then from each sample 0.2 g was digested using 5 cm^3 from the mixture of sulfuric (H_2SO_4) and perchloric ($HCLO_4$) as described by Cottenie *et al.* (1982). Nitrogenase activity was determined on a detached root system in a closed system using the method described by LaRue and Kurz (1973). Subsequently, the nodules on each individual root were separated and counted and the total weight of nodules per plant was recorded. Nodule water contents were measured and expressed as a percentage on a nodule fresh weight basis. Total nitrogen was determined by micro-Kjeldahl method and phosphorus was determined calorimetrically at wavelength 680 nm using spectrophotometer (Spekol) while potassium was determined by using Gallen Kamp flame photometer. Total crude protein % in seeds was calculated by multiplying % total nitrogen by 6.25. Fe, Zn and Mn were measured as described by APHA (1992) using By-Uni Com atomic spectrophotometer solar 969AA atomic absorption spectrophotometer. After harvesting, soil samples from surface down to 30 cm at 15 cm intervals were collected. Available nitrogen in the soil was extracted using 2.0 M KCl and determined by using macro-Kjeldahl method, available phosphorus in the soil was extracted with $NaHCO_3$ 0.5 M at pH 8.5 and determined calorimetrically after treating with ammonium molybedate and stannous chloride at a wavelength 660 nm and available potassium was estimated by extracting soil with 1.0 N ammonium acetate at pH 7.0 using flame photometer as described by Hesse (1971). Available micronutrients (Fe, Mn and Zn) were measured as described by APHA (1992) using By-Uni Com atomic spectrophotometer solar 969AA atomic absorption spectrophotometer.

The data were subjected to one way analysis of variance (ANOVA) appropriate to the randomized complete block design applied after testing the homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). The significant differences between treatments were compared with the critical difference at 5% probability level by the Duncan's test.

RESULTS AND DISCUSSION

Nutrients status in the soil after harvesting of cowpea: No doubt that the agricultural potential of sandy soils depends on the availability of sufficient water for crop cultivation and the provision of nutrients. Table 1 reveals a vital effect of macro and micro nutrients on soil fertility. The superiority residual effect after harvesting was associated with bio fertigation jointly with 50% NPK+HS in drip irrigation followed by drip fertigation of 100% NPK with all macronutrients in soil. The highest values of macronutrients were 60.0, 8.13 and 300 mg kg soil^{-1} for N, P and K, respectively obtained from 50% NPK+HS+BF treatment. Meanwhile, less increase in soil available micronutrients, Fe, Mn and Zn due to the application of bio or organic and inorganic fertilizers than those obtained with the above mentioned macronutrients. Nevertheless, 50% NPK+HS+BF gave the highest values 5.58, 3.04 and 1.50 mg kg soil^{-1} for Fe, Mn and Zn, respectively, compared with the other treatments. It is well known that humic substances increases soil's cation exchange capacity (ability to hold and release cations such as K^+, Ca^{++}, or NH^{4+}) and also can form aqueous complexes with micronutrients, though not to the same extent as many synthetic chelating agents (Mikkelsen, 2005). Since, Humic acid holds cations so they could be absorbed by a plant's root, improving micronutrient exchange and transference to the plant's circulation system (Adani *et al*., 1998).

Cowpea nodulation and N_2 fixation: Nodulation is controlled by a variety of processes, both external (heat, acidic soils, drought and nitrate) and internal (autoregulation of nodulation, ethylene). Nitrogenase activity (acetylene reduction activity) is considered as an index of N_2 fixation. Table 2 reveals that fertigation with 50% recommended NPK combined with bio-fertigation and humic substances resulted in significant enhancement in number of nodules plant^{-1}, dry weight of nodules (mg plant^{-1}) and nitrogenase activity (μm C_2H_4 h^{-1}plant^{-1}) followed by 50 % recommended dose of NPK combined with bio inoculants followed by 100% recommended dose of NPK finally 50% recommended dose of NPK jointly with humic substances under drip fertigation system. There is a relationship (r^2 = 0.703) between dry weight of nodules (mg plant^{-1}) and nitrogenase activity (μm C_2H_4 h^{-1} plant^{-1}). Karem *et al*. (2000) reported highest values of cowpea fresh and dry weight of nodule under biofertilizer

Table 1: Nutrient contents (mg kg soil^{-1}) in soil as influenced by bio fertigation and humic substances under fertigation system after cowpea harvesting

	Macronutrients			Micronutrients		
	N	P	K	Fe	Mn	Zn
Treatments	(mg kg soil^{-1})			(mg kg soil^{-1})		
Before sowing	26.0^d	2.5^d	95^d	2.98^d	1.90^d	0.80^c
100% NPK	57.3^a	7.1^{ab}	298^a	3.15^c	1.88^c	0.84^c
50% NPK + HS	55.0^c	6.0^b	230^c	5.00^b	2.55^b	1.32^{ab}
50% NPK + BF	52.0^{bc}	6.0^c	213^b	3.14^c	1.89^{cd}	0.93^b
50% NPK + HS+ BF	60.0^b	8.1^a	300^a	5.58^a	3.04^a	1.50^a

Means within a column followed by the same letters are not significantly different according to the Duncan Multiple Range Test ($p<0.05$)

Table 2: Nodules parameters in cowpea plant as influenced by bio fertigation and humic substances under fertigation system at 45 days after sowing

Treatments	Nodules No. plant^{-1}	Nodules dry weight plant^{-1} (mg)	Nitrogenase activity (μmC_2H_4 h^{-1}plant^{-1})
100% NPK	42.3^{ab}	321^c	365^b
50% NPK + HS	33.8^b	252^d	301^c
50% NPK + BF	47.0^{ab}	435^b	392^{ab}
50% NPK + HS+ BF	61.1^a	631^a	410^a

Means within a column followed by the same letters are not significantly different according to the Duncan Multiple Range Test ($p<0.05$)

substances provide sites for microflora to colonize, hence bacteria secrete enzymes which act as catalysts, liberating calcium and phosphorous from insoluble calcium phosphate and iron and phosphorous from insoluble iron phosphate. This bacteria are further stimulated to secrete additional enzymes, liberating more calcium, iron and phosphorous, until both the humic acid and bacterial populations are satisfied. In the same way, trace nutrients are also converted into forms more easily used by the plant (Giesler *et al*., 2005). Moreover, Li and Alexander. (1990) and Aseri *et al*. (2008) reported that Rhizobacteria of genera *Azospirillum, Bacillus,* promote nodulation in many legume species. Moreover, addition of *Azospirillum* sp. and *Azotobacter* sp. together resulting in high numbers of nodules and high weight consequently, this is leading to much more nitrogen-fixed resulted in low amount of mineral nitrogen fertilizer and reduction the cost of fertilization and decreasing the pollution level (Sindhu and Badarwal, 2001).

Cowpea plant growth: Combination of microbial inoculums, humic substances and mineral fertilizers provided through drip irrigation shown in Table 3. There are significant effects on all plant growth parameters except for leaf area (cm^2) and moisture content (%) in pods. Drip fetigation with 100% recommended dose of NPK solely give the highest values of plant height, SPAD value, leaf area and branches number except for leaves number $plant^{-1}$.While the lowest value of the same parameters occurred with drip fertigation with 50% recommended dose of NPK combined with bio fertigation except for branches number and leaves number $plant^{-1}$. Bio fertigation and humic substances all together with 50% recommended of NPK injected through drip irrigation system recorded the highest values of pods number, fresh weight of pods, moisture content (%) in pod and seeds number per pod except for dry weight of pods $plant^{-1}$ as compared to the others. The better performance under drip irrigation could be attributed to maintenance of favorable soil water status in the root zone which in turn helped the plants to utilize moisture as well as nutrients more efficiently from the limited wetted area(Phene and Beale, 1976). Moreover, beneficial bacteria such as *Azospirillum* and phosphobacteria colonizing in the rhizosphere region and has the ability to fix nitrogen, solubilize phosphorus and stimulate plant growth (Madhaiyan *et al*., 2004, 2006).

Cowpea yield and its quality: There are also some reports confirmed the positive role of humic substances in promoting plant biomass, stimulation of seed, straw and even direct effect on crop productivity and increases in crop yields (Bama and Selvakumari, 2001; Taha *et al*., 2006). Comparing among all treatments, maximum seed, straw and biomass yields were recorded in drip fertigation with 100% recommended dose of NPK solely (Table 4). Biofertilizer showed significantly higher yield attributes and seed yield of cowpea as compared to control (Sarker *et al*., 2001). In addition, interaction of bio-fertilizer and chemical fertilizer also showed statistically significant difference. While the highest values of harvest index was found with drip fertigation of 50% recommended dose of NPK jointly with bio fertgation. Mineral fertilizers along with microbial inoculants and humic substances gave rather equal yields as compared to mineral fertilizers alone through drip fertigation system. These findings are in agreement with obtained by Mishra *et al*. (2010). The promotion of plant growth due to application of microorganisms and humic substances which stimulate the plant yields may be due to the higher frequency of bio fertigation with the availability of soil moisture which leads to the effective absorption of nutrients and better proliferation of roots which might increase crop yield (Omer *et al*., 2004). Similar trend was found in seed quality parameters i.e., total crude protein% in seeds and weight of 100 seeds (g), the highest total crude protein and 100-seed weight were 27.88% and 20 g mostly associated with joint application of bioinocultans with *Azospirillum, Azotobacter* and *Basillus megatherium*, humic substances and 50% of mineral fertilizers through

Table 3: Plant growth parameters of cowpea plant as influenced by bio fertigation and humic substances under fertigation system at 65 days after sowing

	Parameters				
Treatments	Plant height (cm)	SPAD value	Leaf area (cm^2)	Branches number $plant^{-1}$	Leaves No. $plant^{-1}$
Foliage					
100% NPK	85.6^{a}	45.2^{a}	632.0^{a}	7.6^{a}	47.0^{b}
50% NPK+HS	80.7^{b}	41.3^{bc}	623.0^{a}	6.6^{b}	42.6^{d}
50% NPK+BF	78.0^{c}	40.2^{c}	621.0^{a}	7.0^{a}	47.0^{c}
50% NPK+HS+BF	83.3^{ab}	44.7^{b}	630.0^{a}	7.3^{a}	50.0^{a}
Pods					
100% NPK	41.8^{a}	185.88^{a}	19.58^{ab}	89.47^{a}	11.3^{a}
50% NPK+HS	38.3^{c}	173.59^{b}	21.00^{a}	87.90^{a}	9.8^{b}
50% NPK+BF	36.5^{d}	182.25^{ab}	19.00^{b}	89.57^{a}	9.0^{c}
50% NPK+HS+BF	42.0^{a}	186.58^{a}	19.35^{ab}	89.63^{a}	11.5^{a}

Means within a column followed by the same letters are not significantly different according to the Duncan Multiple Range Test ($p<0.05$)

Table 4: Seed yield, straw, biomass, harvest index, fertilizer use efficiency, 100-seed weight and total crude protein % in cowpea plant as influenced by bio fertigation and humic substances under fertigation system at harvest.

Treatments	Seed yield (to ha^{-1})	Straw yield (to ha^{-1})	Biomass yield (to ha^{-1})	Harvest Index (%)	Fertilizer U. E.% (kg seed kg^{-1} NPK)	100-seed weight (g)	Total crude protein (%)
100% NPK	2.217^{a}	2.366^{a}	4.583^{a}	48.4^{a}	23.7^{c}	12.49^{a}	27.2^{ab}
50% NPK + HS	2.204^{b}	2.345^{b}	4.549^{a}	48.5^{a}	46.9^{b}	10.57^{b}	25.1^{c}
50% NPK + BF	2.205^{b}	2.344^{ab}	4.549^{a}	48.5^{a}	46.9^{b}	11.54^{ab}	26.0^{b}
50% NPK + HS+ BF	2.215^{a}	2.360^{b}	4.575^{a}	48.4^{a}	47.2^{a}	12.51^{a}	27.9^{a}

Means within a column followed by the same letters are not significantly different according to the Duncan Multiple Range Test ($p<0.05$)

Table 5: Chemical composition of seeds and straw as influenced by bio fertigation and humic substances under fertigation system at harvest

	% of dry matter			mg kg^{-1} of dry matter		
Treatments	N	P	K	Fe	Mn	Zn
Seeds						
100% NPK	4.36^{b}	0.152^{a}	3.05^{a}	40.6^{d}	26.0^{c}	10.0^{c}
50% NPK+HS	4.01^{d}	0.126^{a}	3.01^{a}	49.6^{b}	35.9^{b}	15.4^{b}
50% NPK+BF	4.16^{c}	0.122^{a}	2.88^{a}	45.0^{c}	31.3^{bc}	12.1^{c}
50% NPK+HS+BF	4.46^{a}	0.155^{a}	2.98^{a}	60.3^{a}	38.0^{a}	18.4^{a}
Straw						
100% NPK	1.40^{a}	0.032^{a}	2.46^{a}	50.4^{c}	48.0^{c}	39.9^{c}
50% NPK+HS	1.11^{c}	0.026^{a}	2.00^{c}	61.4^{b}	56.9^{b}	45.8^{ab}
50% NPK+BF	1.27^{b}	0.018^{a}	1.91^{d}	52.4^{c}	51.3^{c}	42.0^{b}
50% NPK+HS+ BF	1.37^{ab}	0.033^{a}	2.41^{b}	65.3^{a}	62.6^{a}	52.6^{a}

Means within a column followed by the same letters are not significantly different according to the duncan multiple range Test ($p<0.05$)

drip irrigation system (Table 4). Fertilizer-use Efficiency (FUE) was significantly superior in all the treatments compared to drip fertigation of 100% mineral fertilizer solely (Table 4). This may be due to better availability of treatments. Humic moisture and nutrients throughout the growth stages in bio fertigation system leading to better uptake of nutrients and production of cowpea seeds.

Elemental composition of cowpea plants: Table 5 shows that bio fertigation and humic substances jointly with 50% recommended of NPK added through drip irrigation system recorded the highest N and P in both seeds and straw compared to other treatments. However, drip fertigation of 100% mineral fertilizer solely recorded the highest values of K in both seeds and straw compared to the rest of bio fertigation or/and humic substances treatments. All treatments had significant effect on N and K except for the P% in both seeds and straw. Cowpea plants inoculated with *Azospirillum, Azotobacter* and *Basillus megatherium* and drip fertigated humic substances and 50% recommended dose of NPK recorded the highest values of Fe, Mn and Zn in both seeds and straw tissues, respectively compared with other treatments. Those findings are in agreement with results of Asik *et al*. (2009). The enhancement in nutrents content in seed and straw due to humic substances based on HS provides numerous benefits to crop production. It assists in transferring micronutrients from the soil to the plant, enhances water retention, increase seed germination rates, penetration and stimulates development of microflora populations in soils (Adani *et al*., 1998; Mikkelsen, 2005).

CONCLUSION

It could be concluded that injection of 50% recommended dose NPK as drip fertigation in combination with biofertigation of liquid formulation and humic substances was better and comparable way than the other treatments. Hence, bio fertigation of microbial inoculums and humic substances could be used as a complementary for mineral fertilizers to improve yield and quality of cowpea under sandy soil conditions which protect the environment chemical pollution and its harmful effect on human and animal health.

REFERENCES

APHA, 1992. Standard Methods for the Extraction of Water and Wastewater. 18th Edn., APHA, Washington, USA.

Adani, F., P. Genevini, P. Zaccheo and G. Zocchi, 1998. The effect of commercial humic acid on tomato plant growth and mineral nutrition. J. Plant Nutr., 21: 561-575.

Ahmed, N., M. Rashid and A.G. Vaes, 1996. Fertilizer and their Use in Pakistan. 1st Edn., National Fertilizer Development Centre, Islamabad, Pakistan, pp: 9.

Aseri, G.K., N. Jain, J. Panwar, A.V. Rao and P.R. Meghwal, 2008. Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of pomegranate (*Punica granatum* L.) in Indian Thar Desert. Sci. Hortic., 117: 130-135.

Asik, B.B., M.A. Turan, H. Celik and A.V. Katkat, 2009. Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihli) under conditions of salinity. Asian J. Crop Sci., 1: 87-95.

Bama, K.S. and G. Selvakumari, 2001. Effect of humic acid on growth yield and nutrition of amaranthus. South Indian Hort., 49: 155-159.

Cottenie, A., M. Verloo, L. Kiekens, G. Velghe and R. Camerlynck, 1982. Chemical Analysis of Plants and Soils. Laboratory of Analytical and Agrochemistry, State University-Ghent, Belgium.

Das, D.K. and N. Ram, 2006. The nature of humic substances under long-term manuring and fertilization in a rice-wheat system. Int. Rice Res. Notes, 31: 29-31.

Giesler, R., T. Andersson, L. Lövgren and P. Persson, 2005. Phosphate sorption in alumium- and iron-rich humus soils. Soil Sci. Soc. Am. J., 69: 77-86.

Gomez, K.A. and A.A. Gomez, 1984. Statistically Procedures for Agricultural Research. 2nd Edn., John Wiely and Sons, New York, pp: 680.

Hesse, P.R., 1971. A Text Book of Soil Chemical Analysis. John Murry Publication, London, UK., pp: 384-387.

Hussain, T., T. Javid, J.F. Parr, G. Jilani and M.A. Haq, 1999. Rice and wheat production in Pakistan with effective microorganism. Am. J. Alt. Agri., 14: 30-36.

IITA, 2004. Report of internal midterm evaluation. International Institute of Tropical Agriculture, April 20th-May 1st 2004. http://www.iita.org/.

Karem, H.A., S.M.A. Radwan and M. Attia, 2000. Impacts of certain biofertilizers on cowpea plants in sludge amended sandy soil. Pak. J. Biol. Sci., 3: 423-428.

LaRue, T.A. and W.G.W. Kurz, 1973. Estimation of nitrogenase using a colorimetric determination for ethylene. Plant. Physiol., 51: 1074-1075.

Li, D.M. and M. Alexander, 1990. Factors affecting co inculcation with antibiotic producing bacteria to enhance rhizobial colonization and nodulation. Plant Soil, 129: 195-201.

Madhaiyan, M., S. Poonguzhali, M. Senthilkumar, S. Seshadri and H. Chung *et al*., 2004. Growth promotion and induction of systemic resistance in rice cultivar Co-47 (*Oryza sativa* L.) by Methylobacterium spp. Bot. Bull. Acad. Sin., 45: 315-324.

Madhaiyan, M., B.V. Suresh Reddy, R. Anandham, M. Senthilkumar, S. Poonguzhali, S.P. Sundaram and T. Sa, 2006. Plant growth-promoting Methylobacterium induces Defense responses in groundnut (*Arachis hypogaea* L.) compared with rot pathogens. Curr. Microbiol., 53: 270-276.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd Edn., Academic Press, London, UK., pp: 889.

Mikkelsen, R.L., 2005. Humic materials for agriculture. Better Crops, 89: 6-10.

Mishra, A., K. Prasad and G. Rai, 2010. Effect of bio-fertilizer inoculations on growth and yield of dwarf field pea (*Pisum sativum* L.) in conjunction with different doses of chemical fertilizers. J. Agron., 9: 163-168.

Ngakou, A., D. Nwaga, N.N. Ntonifor, M. Tamo, C.L.N. Nebane, I.A. Parh, 2007. Contribution of arbuscular mycorrhizal fungi (AMF), rhizobia and *Metarhizium anisopliae* to cowpea protection in Cameroon. Int. J. Agric. Res., 2: 754-764.

Omer, Z.S., R. Tombolini, A. Broberg and B. Gerhardson, 2004. Indole-3-acetic acid production by pink-pigmented facultative methylotrophic bacteria. Plant Growth Regul., 43: 93-96.

Phene, C.J. and O.W. Beale, 1976. High-frequency irrigation for water nutrient management in humid regions. Soil Sci. Soc. Am. J., 40: 430-436.

Sarker, P.C., A.F.M.F. Rahman, S.M.A. Shiblee, A. Muqit, A.S.M.M. Uddin and M.N. Anwar, 2001. Effect of *Rhizobium Inoculants* on cowpea under rainfed condition. Pak. J. Biol. Sci., 4: 654-656.

Sindhu, S.S. and K.R. Badarwal, 2001. Chitinolytic and cellulolytic *Pseudomonas* sp. Antagonisitic to fungal pathogens enhances nodulation by *Mesorhizobium* sp. Cicer in chickpea. Microbial. Res., 156: 353-358.

Taha, A.A., A.S. Modaihsh and M.O. Mahjoub, 2006. Effect of humic acids on wheat plant grown on different soils. J. Agric. Sci. Mansoura Univ., 31: 4031-4039.