Response of damssisa to biofertilizers and organic manure application in presence of *Pseudomonas fluorescens*

Zaghloul, R. A. [1] ; T. M. El-Husseiny [1]; Ehsan A. Hanafy[1]; A. GH. Rahal [2] and H. M. , Abdelrahman.[1]

1- Fac. Agric., Moshtohor, Benha University, Egypt.

2- Soil, Water and Environment Research Inst. Agric. Research Center, Giza, Egypt.

ABSTRACT

Two field experiments were carried out at an organic farm during 2005- 2006 and 2006-2007 seasons. The aim of this research is studying the response of damssisa to biofertilization and compost amendment in presence of *Pseudomonas fluorescens*. Obtained data showed significant increase of nitrogenase and phosphatase activity with dual of biofertilization and organic manuring rather than each one individually.

Moreover, ammoniacal, nitrate nitrogen and available phosphorus were the highest records with dual application of biofertilizers and compost.

Generally, compost amendment gave higher records of growth characteristics and herb yield compared with biofertilizers inoculation.

Significant increase was observed in studied growth characteristics and herb yield of damssisa under dual application of biofertilizers and compost in presence of *Ps. fluorescens*.

key words: Damssisa, biofertilization, organic manuring, *Ps. fluorescens*, nitrogenase, phosphatase activity and damssisa herb yield.

INTRODUCTION

Damssisa is used in Egyptian folk medicine as remedy of rheumatic pains, decoction of plant for asthma, Bilharziasis , diabetes and to expel kidney stones. Nosering branches stimulant, stomachic, slightly astringent , emollient, vulnerary, diuretic , in renal troubles and expel renal stones (**Amin, 1990**).

Production of medicinal and aromatic plants by using dual application of biofertilization and organic manuring became an essential process to ensure the safety, not only for human, but also for the environment in which we live. This type of farming is required to substitute of chemical fertilizers for healthy and good quality of production.

The increasing of yield can easily be demonstrated with mycorrhizal inoculation since it enhance of vegetative growth and plant fitness (**Johnson *et al*, 1997**). Mycorrhizal fungi may also improve soil quality by having a direct influence on soil aggregation, aeration and water dynamics (**Rillig, 2004**).

Khalil (2006) studied the effect of farm yard manure, poultry and commercial compost combined with *Azotobacter chroococum* on *Plantago afra*. The obtained results indicated that application of *A. chroococum* pronouncedly improved the effect of organic fertilization on growth characteristics, yield and N, P and K percentage in herb.

Ponmurgan and Gopi (2006) found that positive correlation between phosphate solubilizing bacteria and phosphatase activity.

Abdelaziz *et al* (2007) reported that the plants treated by a mixture of compost and biofertilizers showed a significant increase in vegetative growth, total N, P and carbohydrate content and essential oil production for rosemary. The findings clearly indicate that compost and biofertilizers could be replace conventional NPK fertilizers in the cultivation of rosemary and consequently minimize environmental pollution by these compounds.

Nitrogenase activity (N_2-ase) was used as a criterion of atmospheric nitrogen fixation by diazotrophs. **Zaghloul *et al* (2007)** indicated that inoculation with *Azotobacter chroococcum* individually or in combination with biocontrol agents of *Trichoderma harzianum* and *Bacillus subtilis* significantly increased N_2-ase activity compared to un-inoculated treatments.

Therefore, this research was carried out to study the various treatments of organic farming in the form of combination between organic manure, biofertilizers and biocontrol agent and their effects on some enzymes activity, damssisa growth characteristics and herb yield.

MATERIALS AND METHODS

Two field experiments were carried out at an organic farm, GHAREEB SONS FARMS, in Minoufia Governorate, Egypt, during 2005/ 2006 and 2006/2007 seasons. This experiment was designed to study the response of damssisa to biofertilization and compost amendment in presence of *Pseudomonas fluorescens*.

Seeds of damssisa were obtained from Medicinal and Aromatic Research Dept., A.R.C., Ministry of Agriculture.

Experimental soil

Soil samples obtained from different field places were mixed. Soil samples were subjected to physical and chemical analyses as follows. Mechanical and chemical analyses are presented in Table (1).

Table1. Mechanical and chemical analysis of the experimental soil.

Particle size distribution		**Coarse sand %**	**6.59**	**Textural class**
		Fine sand %	**27.64**	
		Silt %	**12.60**	**Clayey**
		Clay %	**53.17**	
pH			**8.03**	
E.C. (ds/m)			**2.02**	
Organic matter %			**2.12**	
Soluble cations meq / l		**Ca^{++}**	**12.1**	
		Mg^{++}	**5.8**	
		Na^{+}	**0.44**	
		K^{+}	**1.86**	
Soluble anions meq / l		**HCO_3^{-}**	**4.40**	
		$CO_3^{=}$	**0**	
		Cl^{-}	**11.2**	
		$SO_4^{=}$	**4.6**	
Total and available macronutrients (ppm)	**N**	**total**	**1730**	
		available	**53.64**	
	P	**total**	**561.2**	
		available	**130.94**	
	K	**total**	**3500**	
		available	**1612.8**	

The main characteristics of used compost which obtained from organic farm in Minoufia Governorate, GHAREEB SONS FARMS, are given in Table (2).

Table 2. Chemical analysis of the used compost in this experiment.

Parameters	**Unit**	**Value**
pH	-	8.11
EC (1:5 extract)	ds/m	8.21
Organic matter	%	21.57
Organic-C	%	12.54
Total-N	%	1.21
C/N ratio	-	10.36
Total-P	%	0.91
NH_4- N	ppm	274.7
NO_3-N	ppm	50.1

Mechanical and chemical analyses of soil and compost were carried out according to the method described by **Page *et al* (1982)**.

Experimental design

Treatments were distributed in a randomized complete block design with three replicates. The experimental area was 10.5 m^2 (3 x 3.5 m). This experiment included the following treatments:

T1: Control (non-fertilized and non-inoculated).

T2: Chemical fertilization (recommended doses of chemical fertilization N,P and K).

T3: Biofertilization (*Azotobacter chroococcum* + *Glomus macrocarpum* + *Bacillus circulans*).

T4: Biofertilization + half dose of compost.

T5: Biofertilization + 3/4 dose of compost.

T6: Biofertilization + recommended dose of compost.

T7: Recommended dose of compost (8 ton/fed).

The biocontrol agent of *Pseudomonas fluorescens* was added to all investigation treatments

Biofertilizers and biological control agents strains

A. chroococcum, Bacillus circulans and *G. macrocarpum* were kindly obtained from Department of Agricultural Microbiology, Soil, Water and Environmental Research Inst., Agric. Research Center, Giza, Egypt.

Ps. fluorescens was isolated and identified by staff members of Agricultural Microbiology, Fac. Agric. Moshtohor, Banha Univ., Egypt.

Inocula preparation

Inocula of *A. chroococcum*, *B. circulans* and *Ps. fluorescens* were prepared in modified Ashby's medium, Alexandrov and King's broth medium according to **Abdel-Malek and Ishac, 1968, Zahra, 1969 and king *et al*, 1954** respectively.

For preparation of *Glomus macrocarpum* inoculum, pots of 30 cm in diameter were filled with autoclaved sandy loam soil. The soil of each pot was inoculated with VAM fungus *G. macrocarpum.* Five onion seedlings were transplanted in each pot as a host plant. After 14 weeks, spores of VAM were collected from rhizosphere and roots of onion and extracted by wet sieving (**Gerdemann** and **Nicolson, 1963**). VAM spores were counted by the method described by **Musandu** and **Giller (1994).**

Cultivation process

Prior to cultivation, plant bed was prepared. The seeds of damssisa were sown in prepared seed beds on 10th September for both study seasons. After 50-60 days seedlings 10-15 cm length were individually transplanted in experimental field.

Chemical fertilizers were supplemented with full dose of inorganic nitrogen (50 kg N/fed) as ammonium sulphate, inorganic phosphorus (25 kg P_2O_5/fed) as super phosphate and potassium (40 kg K_2O/fed) as potassium sulphate .The chemical fertilizers were applied in two equal doses *i.e.* at vegetative and flowering stages.

Compost was added as organic manure to the soil at a rate of recommended dose (8 ton/fed), 1/2 dose and 3/4 dose before planting.

Biofertilizers and biocontrol agents application

Except for control treatment, transplants of damssisa seedlings were soaked by dipping the root system in mixture of sucrose solution (40 %) as an adhesive for inocula, and cell suspension of each *Azotobacter chroococcum* ($3x10^{11}$ c.f.u./ml)), *B. circulans* ($4.1x10^{11}$ c.f.u./ml) and *Ps. fluorescens H2* ($9.6x10^{11}$ c.f.u./ml) for the same time mentioned above. The same prepared inocula were added to the plots three times (every month) throughout the growing season at a rate of 100 ml. pot^{-1}. Regarding the mycorrhizal treatments, *Glomus macrocarpum* strain was used at the rate of 20 ml (160 spores/ml)/ plot.

Microbiological analysis

Nitrogenase and phosphatase were estimated in rhizosphere soil samples at 30, 60, 90 and 150 day from planting. Nitrogenase was determined by the method described by **Okafor** and **MacRae, 1973).** Phosphatase was assayed according to **Drobnikova (1961)**,

Macronutrients availability

Available nitrogen, phosphorus and soluble potassium were estimated in rhizosphere at 30, 60, 90 and 150 days from planting. NH_4-N and NO_3- N were determined according to the method described by **Bremner** and **Keeny (1965).** Available phosphorus was determined according to the method described by **Watanabe** and **Olsen (1965).**

Growth characteristics and yield

Plant height (cm), number of branches, dry weight of plant (gm) and dry weight of herb yield/fed were determined.

Statistical analysis

Obtained data were statistically analyzed according to **Gomez** and **Gomez (1984)**. For comparison between means, **Duncan's multiple range test** was used **(Duncan , 1955)**.

RESULTS AND DISCUSSIONS

Periodical changes of nitrogenase activity under biofertilization and/or organic manuring in soil cultivated with damssisa.

Data in **Fig (1)** showed that N_2-ase activity was affected by the investigated treatments. It is not a surprising result that, N_2- ase activity decreased in soil amended with chemical fertilizer compared with inoculated and composted soil.

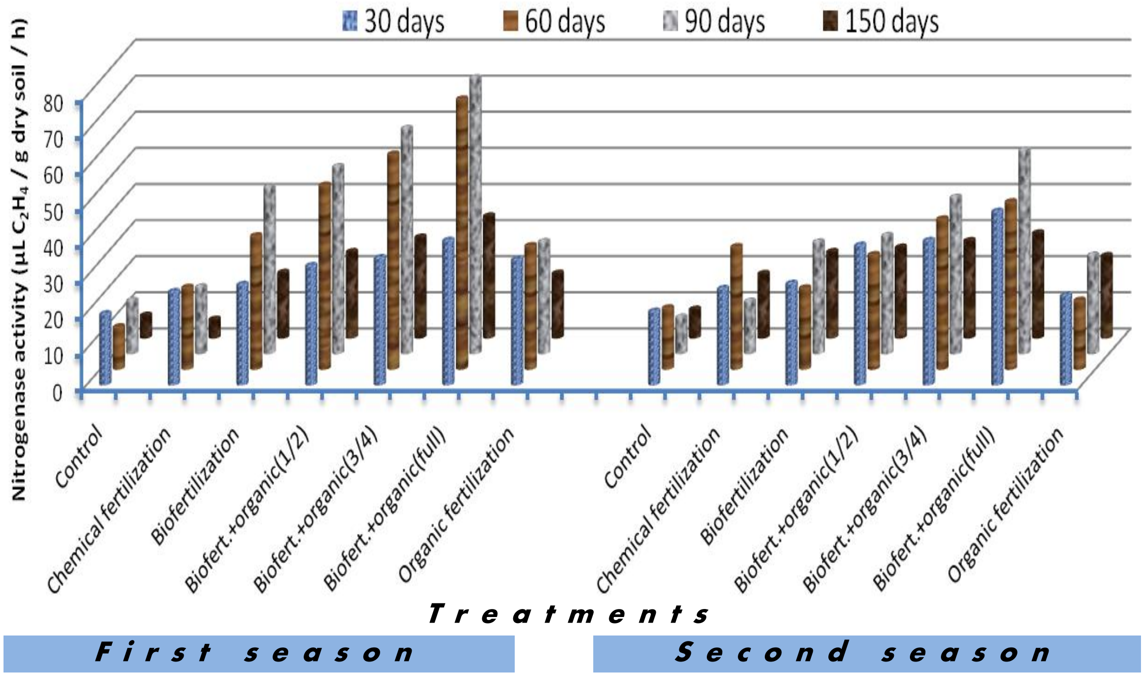

Fig 1. Periodical changes of nitrogenase activity under biofertilization and/or organic manuring in soil cultivated with damssisa.

This was true in the two growing seasons. Lower values of N_2-ase activity may be due to the inhibition of N_2-fixers with the amendment of inorganic nitrogen fertilizer at a high rate. This result is in agreement with **Bisseling *et al*, 1978 and Anne-Sophie *et al,* 2002** who found that the addition of inorganic N-fertilizers decreased the nitrogenase activity.

Higher records of N_2-ase activity were observed in inoculated soil with the biofertilizer strains (*A. chroococcum, G. macrocarpum* and *B. circulans*) than treated soil with compost only. This result externalized the importance of inoculation on proliferation and enhancement of N_2-fixers in rhizosphere.

Also, obtained data showed that treated soil with compost in combination with biofertilization gave higher values of N_2-ase activity than that treated with each one solely. Enhancement of biological activities caused by organic manure might be due to the introducing of large amount of living microorganisms and readily utilizable carbon sources on which microorganisms live. Similar trend of results was observed at all determination periods.

Soil inoculation and amended with full dose of compost showed significant increase in N_2-ase activity compared by amended soil with $^3/_4$ or $^1/_2$ dose of compost. Therefore, N_2-ase activity was increased with the increasing of compost dose. Similar trend of results was observed in both growing seasons. These results are in harmony with those obtained by

Cheng and **Zhiping (2007)** who found that when the amount of compost application was increased, the soil enzymes activities were increased.
The highest values of N_2-ase activity were obtained with the full dose of compost.

As a result of continuous addition of the biofertilizers during the growth season, obtained data revealed that values of N_2-ase activity in inoculated soil were higher than un-inoculated one. This result explains the synergistic effect of inocula addition on survival and activities of beneficial microorganisms.

It's worthily to mention that N_2-ase activity values were higher at flowering stage (90 days from transplanting) rather than vegetative one. Higher records of N_2-ase activity at flowering stage could be attributed to the beneficial effect of root exudates which increase during this stage of cultivated plants.

This result is in harmony with those obtained by **Neweigy *et al* (1997)** and **Hanafy *et al* (1998)** who found that the densities of N_2-fixing bacteria in rhizosphere were higher at heading (flowering) stage of plant growth rather than other plant growth stages. Also, **Aulakh *et al* (2001)** reported that the exudation rates were in general lowest at seedling stage, increased until flowering but decreased at maturity.

Periodical changes of Phosphatase activity under biofertilization and/or organic manuring in soil cultivated with damssisa.

Data in **Fig (2)** showed that the records of phosphatase activity were widely affected with organic manure and biofertilization application.

Results showed that phosphatase activity in the treatment of soil amended with compost was significantly higher than that amended with chemical fertilizers. This result is in agreement with that observed by **Martens *et al* (1992)** who reported that the addition of organic matter maintained high levels of phosphatase activity in soil during a long term study. **Gius-quiani *et al* (1994)** reported that phosphatase activity increased when compost was added to the soil.

Also, **Rosa *et al* (2006)** and **Krishnakumar *et al* (2007)** noticed that recommended chemical fertilizer significant decrease the phosphatase activity than all organic manure treatments.

Cheng and Zhiping (2007) reported that the activity of soil microbial enzymes are greatly stimulated by the addition of organic manure.

Obtained data emphasized that inoculation of soil with effective biofertilizer strains led to significant increase in phosphatase activity compared to soil amendment with compost without inoculation. This result externalized the beneficial effect of biofertilizer strains on phosphatase activity.

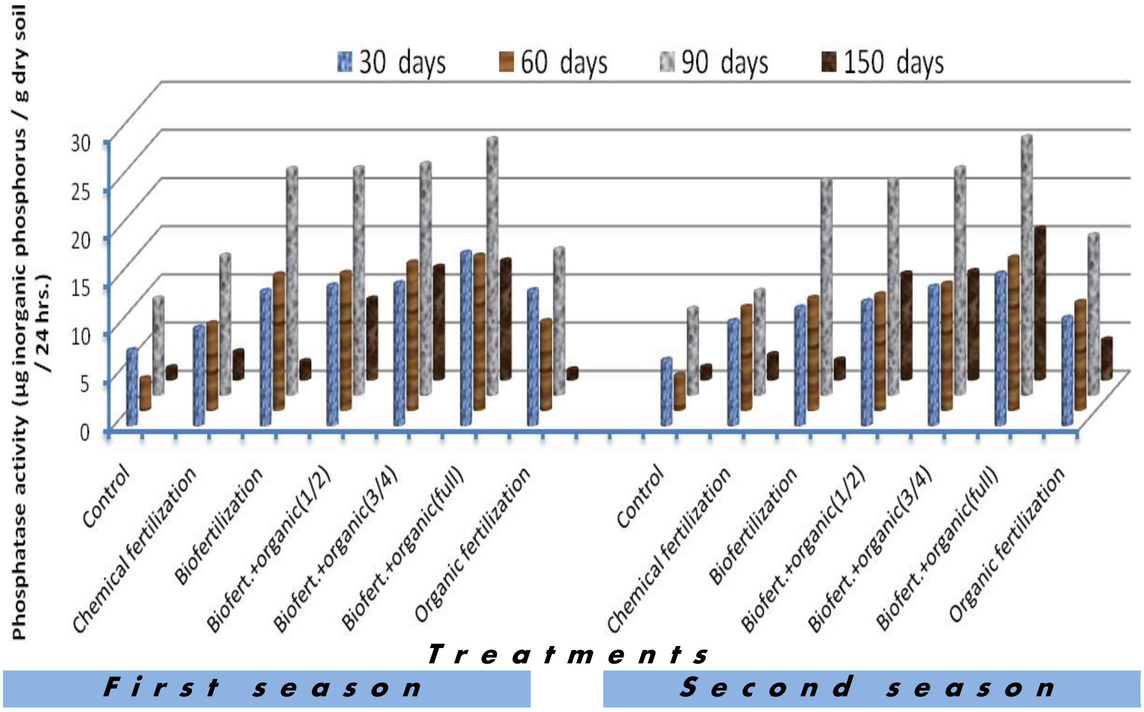

Fig 2. Periodical changes of phosphatase activity under biofertilization and/or organic manuring in soil cultivated with damssisa.

Also, phosphatase activity was enhanced in the treatment of inoculated soil and treated with compost compared to treated soil with either biofertilizers or compost each one individually. This result is likely be due to the efficiency of biofertilizers on phosphatase activity as well as the beneficial effect of compost as nutritional substances for stimulating different soil microorganisms specially P-solubilizers. This result is in accordance with **Balakrishnan *et al*, 2007** who found that the application of compost in combination with phosphate solubilizing bacteria significantly increased soil microflora such as bacteria, fungi and actinomycetes and soil enzyme activities such as dehydrogenase and phosphatase.

The highest records of phosphatase activity were observed in soil treated with full dose of compost in combination with biofertilizers inoculation. Records of phosphatase activity were higher at flowering stage. This result can be attributed to the positive effect of the root exudates of cultivated plants during flowering stage.

Periodical changes of ammoniacal nitrogen under biofertilization and/or organic manuring in soil cultivated with damssisa.

Data presented in **Fig (3)** showed that ammoniacal nitrogen (NH_4-N) records in rhizosphere of damssisa were significantly increased under the investigated treatments compared with the control. Similar trend of results was obtained with both growing seasons.

Soil amended with chemical fertilization gave lower values of NH_4-N compared with the soil inoculated with biofertilizer strains and amended with the organic manure.

Furthermore, inoculated soil with the biofertilizer strains in combination with compost showed higher NH_4-N records than treated soil with either biofertilizers or compost each one individually. The higher records of NH_4-N in case of inoculated and manured soil is likely be due to the positive effect of compost with its high organic nitrogen content which is converted through microbial metabolism into readily usable ammoniacal and nitrate nitrogen.

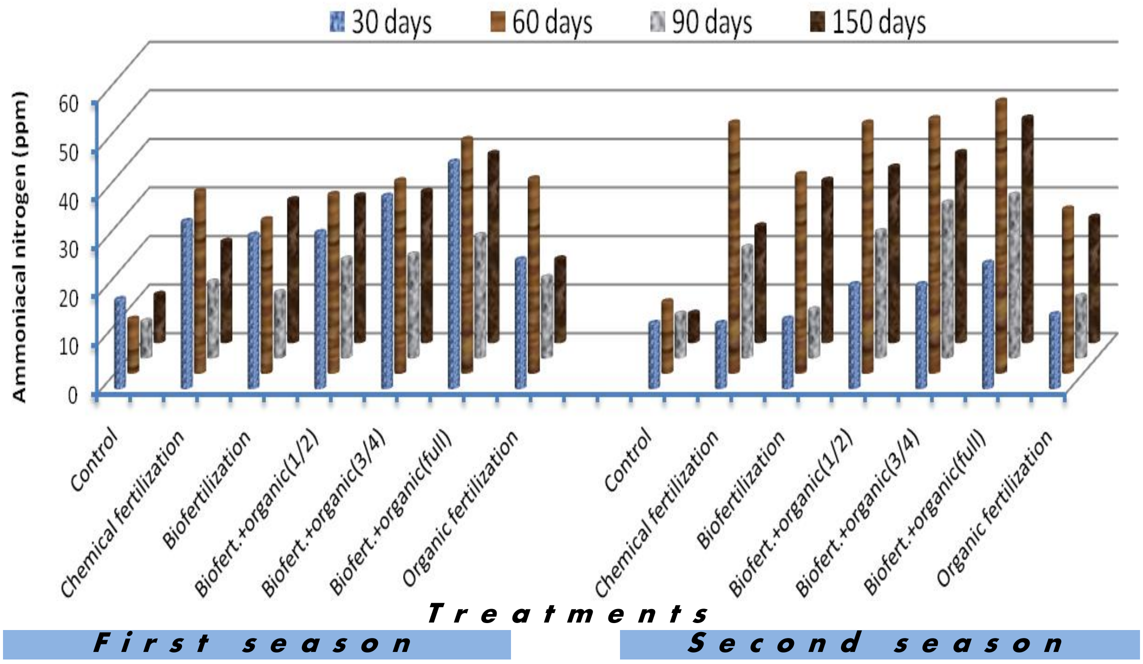

Fig 3. Periodical changes of ammoniacal nitrogen under biofertilization and/or organic manuring in soil cultivated with damssisa.

Organic manures are relatively slow acting and supply variable nitrogen ions for a longer periods. Besides, the positive role of biofertilizer strains on N_2-fixation and ammonification processes.

Similar results were observed by **Yin-Po and Cben-Ching (1995)** who mentioned that application of organic farming practices improved chemical, physical and biological properties of soil than conventional farming.

Data showed that inoculated soil with the biofertilizer strains gave lower values of NH_4-N than soil amended with full dose of compost.

It is important to mention that increasing of organic manure dose led to increase of NH_4-N levels.

Also, the highest values of NH_4-N were observed in soil treated with biofertilizer strains and full dose of compost. This result confirms the importance of inoculation with biofertilizers on microbial society and their activities in the rhizosphere

From the obtained data it is important to notice that the records of NH_4-N were higher in initial period and decreased thereafter. The decrease of NH_4-N levels were observed at flowering stage.

This phenomena can be attributed to the high multiplication of nitrifiers which convert ammoniacal nitrogen to nitrate nitrogen during flowering stage as a result of qualitative and quantitative changes in nature of the root exudates of cultivated plants during different growth stages.

Periodical changes of nitrate nitrogen under biofertilization and/or organic manuring in soil cultivated with damssisa.

Data in **Fig (4)** showed higher NO_3-N levels in inoculated soil with biofertilizer strains and manured with compost than soil treated with either biofertilizers or compost each one individually. The higher values of NO_3-N are likely be due to the beneficial effect of compost and biofertilizers on N_2-fixation and nitrification processes.

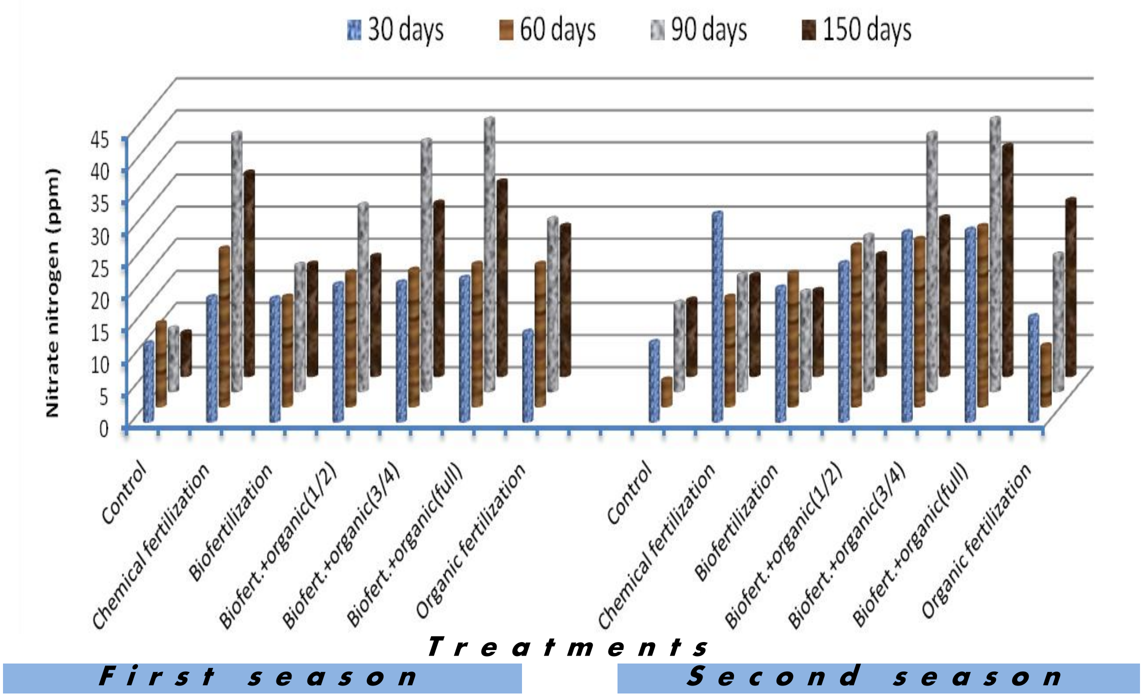

Fig 4. Periodical changes of nitrate nitrogen under biofertilization and/or organic manuring in soil cultivated with damssisa.

Similar trend of results were observed by **Rosa *et al* (2006)** who reported that diversity of ammonia oxidizing bacteria was higher in the compost soils.

It is worthily to mention that the amendment of compost in combination with the biofertilizers showed higher records of NO_3-N compared with the treatment of the soil amended with compost only.

Obtained data showed that inoculated soil with the biofertilizer strains gave higher values of NO_3-N than amended soil with the full dose of compost. This result was contrary to that obtained from soil NH_4-N content. This result could be attributed to the oxidation of ammoniacal nitrogen into NO_3-N by nitrifying bacteria.

NO_3-N records were increased with the increasing of compost amendment. This result confirmed by the increasing of NO_3-N levels in the treatment of amended soil with ¾ dose compost than that amended with ½ dose.

Moreover, the highest values of NO_3-N were observed in inoculated soil and manured with full dose of compost. This result was observed in the two growing seasons.

Concerning the trend of NO_3-N levels during experimental periods obtained data showed that the higher records of NO_3-N were observed at flowering stage. The higher records at flowering stage as a result of the oxidation of NH_4-N to NO_3-N which was observed in this experiment (Fig, 3).

Periodical changes of available phosphorus under biofertilization and/or organic manuring in soil cultivated with damssisa.

Data presented in **Fig (5)** showed that the amended soil with chemical fertilizer gave lower records of available phosphorus compared with inoculated soil with biofertilizer strains and amended with full dose of compost.

Also, obtained data showed that available phosphorus concentration significantly decreased in the treatment of inoculated soil with biofertilizer strains compared with the amended soil with full dose of compost.

Concerning the combination between biofertilizers and compost, dual treatment showed higher records of available phosphorus than treated soil with either biofertilizers or compost singularly. The higher values of available-P in case of soil inoculated and fertilized with compost is likely be due to the beneficial effect of compost on multiplication rate of phosphate dissolving bacteria. Moreover, it supplies of major nutrients (N, P, K, Ca, Mg and S) necessary for plant growth. Similar trend of results was observed by **Brandjes *et al* (1996).**

Moreover, it increases the supply of available nitrogen for plant growth and stimulating microbial growth. Accompanying this increased microbial biomass resulted in increased capacity to mineralize organic P and thus to increase available P (**Marinari *et al*, 2000**).

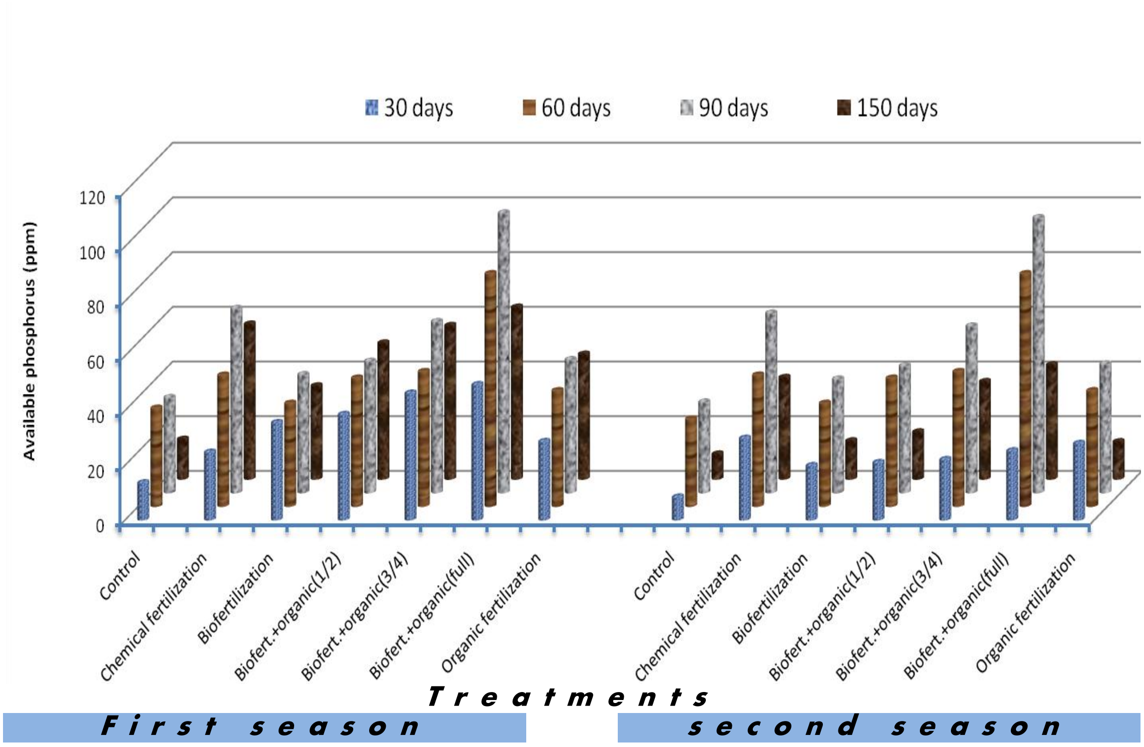

Fig 5. Periodical changes of available phosphorus under biofertilization and/or organic manuring in soil cultivated with damssisa.

Also, **Takeda *et al* (2009)** reported that soil phosphatase activity and phosphate solubilizing bacteria were enhanced in treatments with compost.

Availability of phosphorus through mineralization can be estimated by phosphatase activity (**Tabatabai, 1994**) and phosphate solubilizing bacteria in soil which is considered an important source of phosphorus for plant uptake in P-fixing soils (**Ayaga *et al*, 2006**)

Available phosphorus was increased with the increasing of compost amendment. In this respect, results showed that highly significant increase in available phosphorus values occurred during the two growing seasons.

The highest values of available phosphorus were observed in inoculated and manured soil with full dose of compost.

Effect of biofertilization and/or organic manuring on growth characteristics and herb yield of damssisa.

Data in **Fig (6)** indicated that growth characteristics of damssisa i.e. plant height ,number of branches, herb dry weight per plant and herb yield per feddan were significantly increased under investigated treatments compared to the control in the two growing seasons.

Generally, significant increases were observed in most plant growth characteristics and herb yield with compost amendment treatment compared to biofertilizers inoculation.

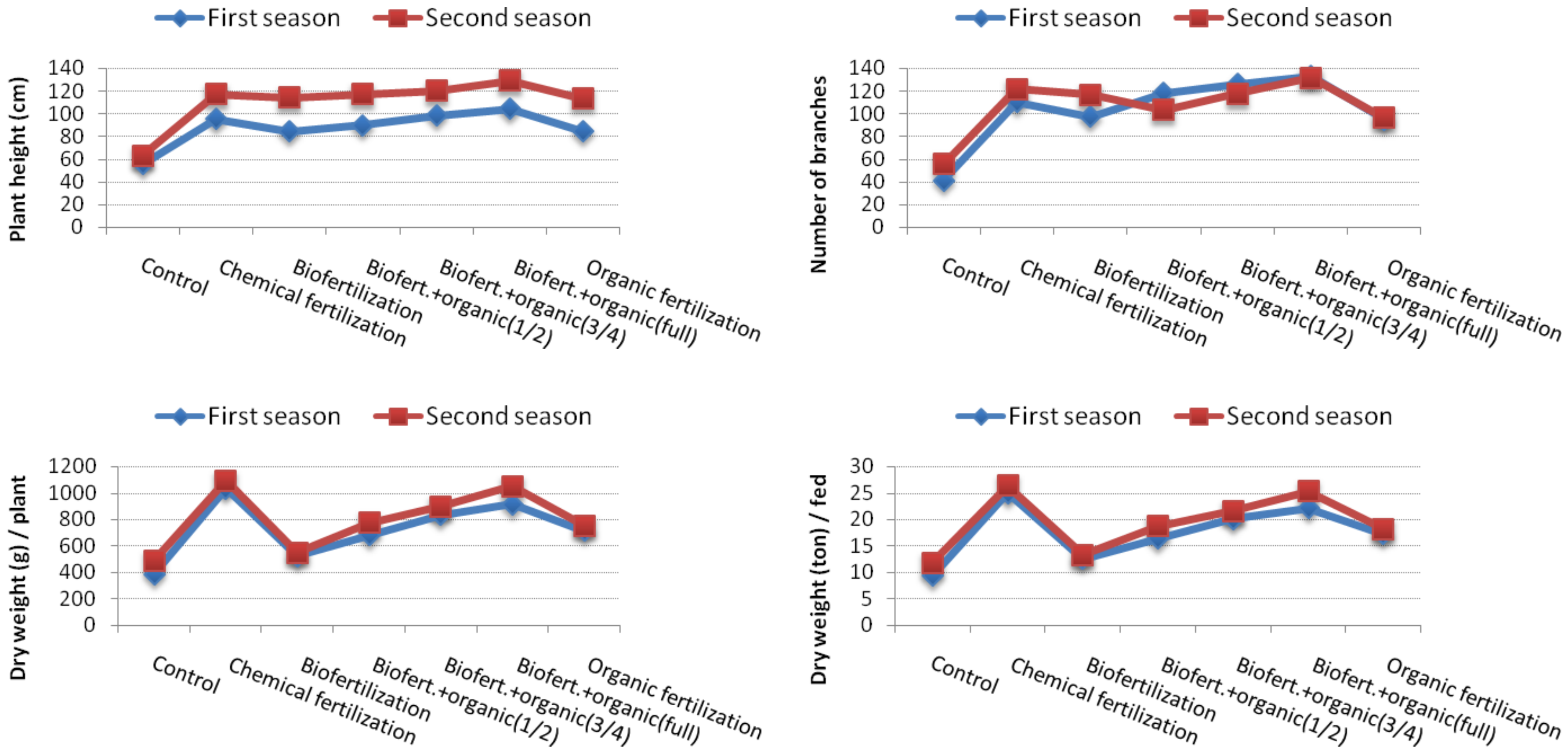

Fig 6. Effect of biofertilization and/or organic manuring on growth characteristics and herb yield of damssisa.

Dual treatment of damssisa with biofertilizer strains and compost gave higher records of growth characteristics and herb yield than the application of each one singularly. This result can be attributed to integrated effect of dual application on all previous parameters which led to improve growth characteristics and herb yield. These results are in accordance with those obtained by **Gharib *et al* (2008)** who reported that the use of combined treatment of biofertilizers and organic manure gave the best results for growth characteristics and herb yield than those obtained from either biofertilizers or organic manure alone.

Concerning the effect of chemical fertilization, data showed that significant increase in growth characteristics and herb yield compared to inoculated damssisa with biofertilizers or manured with full dose of compost.

Respecting the interaction effect between the biofertilizers and compost amendment, data showed that dual application gave significant increase in growth characteristics and herb yield with increasing compost doses. This result could be attributed to the increasing dose of compost that led to increase nutrients availability and activity of beneficial microorganisms.

The highly significant increase in damssisa growth characteristics and herb yield was observed in the treatment of damssisa inoculated with the biofertilizers and manured with full dose of compost. These results could be attributed to the high levels of N_2-ase activity, NH_4-N, NO_3-N and available phosphorus which observed in inoculated damssisa with biofertilizers and manured with full dose of compost.

Conclusion and recommendation

In view of the obtained results, it can be concluded that the dual application of biofertilization and organic manuring had great effect on growth performance and yield of damssisa. As well as, maximum activity of nitrogenase and phosphatase were observed. According to obtained results, it may be recommended that the use of both biofertilization and organic manuring together can substitute chemical fertilization to obtain high productivity of damssisa. In addition, to obtain safety plant and reduce environmental pollution

REFERENCES

Abdelaziz, M. E.; Hanafy, A. H.; Shaaban, M. M. and Pokluda, R. (2007).Fresh weight and yield of lettuce as affected by organic manure and bio-fertilizers. Conference of organic farming, Czech Univ. Agric.,Czech Republic, pp 212-214.

Abdel-Malek,Y. and Ishac, Y.Z. (1968). Evalution of methods used in counting of *Azotobacter* .J. of Appl. Bacteriology, 31: 267-275.

Amin, W. M. A. (1990) A pharmacognostical study of certain Egyptian molliscicidal plant. Ph.D. Thesis, Fac. Pharmacy. Cairo Univ. Egypt.

Anne-Sophie, V.; Christophe, S.; Nathalie, G. M. and Bertrand, N. (2002). Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.).Plant and Soil, 243: 31–42.

Aulakh, M.S.; Wassmann, R.; Bueno, C.; Kreuzwieser, J. and Rennenberg, H. (2001). Characterization of Root Exudates at Different Growth Stages of Ten Rice (*Oryza sativa* L.) Cultivars. Plant boil., 3: 139-148.

Ayaga, G., Todd, A., Brookes, P.C., (2006). Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. Soil Biol. Biochem., 38: 81-90.

Balakrishnan, V. ; Venkatesan, K. and Ravindran, K.C. (2007). The influence of halophytic compost, farmyard manureand phosphobacteria on soil microflora and enzyme activities Plant Soil Environ., 53(4): 186–192.

Bisseling T.; Bos R. C. and Kammen A. (1978). The effect of ammonium nitrate on the synthesis of nitrogenase and the concentration of leghemoglobin in pea root nodules induced by *Rhizobium leguminosarum*. Biochim. Biophys. Acta., 13; 539(1):1-11.

Brandjes, P.J.; de Wit, J.; van Keulen, H. and van der Meer, H.G. (1996). Environmental impact of animal manure management. International Agriculture Centre, Wageningen, The Netherlands.

Bremner, J.M. and Keeny, D.R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. Annals chem. Acta, 32: 485-494.

Cheng, H. and Zhiping C. (2007). Size and Activity of the Soil Microbial Biomass and Soil Enzyme Activity in Long-Term Field Experiments.World Joumal of Agricultural Sciences 3(1): 63-70.

Drobnikova, V. (1961). Factors influencing the determination of phosphatase in soil. Folia. Microbiol., 6: 260.

Duncan, D. B. (1955). Multiple range and multiple "F. test" Biometrics, 11: 1-42.

Gerdemann, J.W. and. Nicolson, T.H. (1963). Spores of mycorrhizal endogenous species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society, 46: 234-244.

Gharib, F.A.; Moussa, L.A. and Massoud, O.N. (2008). Effect of compost and bio-fertilizers on growth, yield and essential oil of sweet marjoram (Majorana hortensis) plant. International Journal of Agriculture and Biology, 10(4): 381-387.

Gius-quiani, P.L.; Gigliotti, G. and Businelli, D. (1994). Long-term effects of heavy metals from composted municipal waste on some enzyme activities in a cultivated soil. Biol. Fert. Soils, 17: 257-262.

Gomez, K.A. and Gomez, A.A. (1984). Statistical procedures for agricultural research. A Wiley-Interscience Publication, John Wiley & Sons, New York .

Hanafy, Ehsan, A.; Neweigy, N. A; Zaghloul, R. A. and El-Sayeda, H. El-Badawy (1998): Inoculation efficiency of rice plants with *Azolla* as a biofertilizer in the presence of different levels of phosphorus. Arab. Univ. Journal Agric. Sci., Ain Shams Univ., Cairo, 6 (1): 49-76.

Johnson, N.; Graham, J. and Smith, F.(1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol., 135: 575–585.

Khalil, Mona, Y. (2006) How-far Would *Plantago afra* L. Respond to Bio and Organic Manures Amendements. Research Journal of Agriculture and Biological Sciences, 2(1): 12-21.

King, E.O., Ward, M.K., Ranney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44: 301–307.

Krishnakumar, S. ; Saravanan, A. ; Natarajan, S.K. ; Veerabadran, V. and Mani, S. (2007) Microbial population and enzymatic activity as influenced by organic farming. Research Journal of Agriculture and Biological Sciences, 1(1): 85-88.

Marinari, S.; Masciandaro, G.; Ceccanti, B. and Grego, S. (2000). Influence of organic and mineral fertilizers on soil biological and physical properties. Bioresoruce Technol., 72: 9-17.

Martens, D. A.; Johanson, J. B. and Frankenberger, W. T. (1992). Production and persistence of soil enzymes with repeated addition of organic residues. Soil Sci., 153: 53-61.

Musandu, A .A. O. and Giller, K. E. (1994). Effect of vesicular-arbuscular mycorrhiza on kubzu (*Pueraria phaseoloides*) growth in phosphate fixing Kenya soils. African crops Sci. J., 2(3): 285-290.

Neweigy, N.A.; Ehsan, A. Hanafy; Zaghloul, R.A. and El-Sayeda, H. El-Badawy (1997): Response of sorghum to inoculation with *Azospirillum*, organic and inorganic fertilization in the presence of phosphate solubilizing microorganisms. Annals of Agric. Sci., Moshtohor, 35 (3): 1383-1401.

Okafor, N. and MacRae, I.C. (1973). The influence of moisture level, light, aeration and glucose upon acetylene reduction by a black earth soil. Soil Biol. Biochem., 5: 181-186.

Page, A. L.; Miller, R. H. and Keeney (1982). Methods of soil analysis. Part 2, 2nd Ed.,Am. Soc. Agronomy, Inc. Mad. Wisconsin, USA.

Ponmurgan, P. and Gopi, C. (2006). In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria . African J. Biotechnology, 5(4): 348-350.

Rillig, M.C. (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. Can. J. Soil Sci.,84: 355–363.

Rosa, M.; Pascuala, J.A.; Garciaa, C.; Hernandeza, M.T. and Insamb, H. (2006). Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biology & Biochemistry, 38: 3443-3452.

Tabatabai, M.A., (1994). Soil enzymes. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdiecek, D., Smith, S., Tabatabai, A., Wollum, A., Mickelson, S.H., Bigham, J.M. (Eds.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. SSSA Book Series 5. SSSA, Madison, WI, pp. 775-833.

Takeda, M. ; Nakamoto, T.; Miyaza, K.; Murayama, T. and Okada, H.(2009). Phosphorus availability and soil biological activity in an Andosol under compost application and winter cover cropping. Applied Soil Ecology, 42 : 86-95

Watanabe, F. S. and Oleson, S. R. (1965). Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Amr. Proc., 29: 677-678.

Yin-Po, W. and Cben-Ching, C. (1995). The effect of organic farming practices on the chemical, physical and biological properties of soil in Taiwan. FFTC book series No. 46. pp. 33-39.

Zaghloul, R.A. ; Hanafy, Ehsan, A.; Neweigy, N.A. and Khalifa, Neamat, A. (2007). Application of biofertilization and biological control for tomato production. Proccedings of 12th Microbiology Conf., Cairo, Egypt. 18-20 March (2007).

Zahra, M. K. (1969). Studies of silicate bacteria. M.Sc. Thesis, Fac. Agric., Cairo Univ., Egypt, p. 111.

أستجابة نباتات الدمسيسة للتلقيح بالسماد الحيوي والعضوي في وجود السيدوموناس فلورسينس

راشد عبدالفتاح زغلول[١]، طلعت محمد الحسيني[١] ، إحسان أحمد حنفى[١] ، أحمد غنيم رحال[٢] ، هاني محمد أحمد عبدالرحمن[١]

١ – كلية الزراعة– جامعة بنها. ٢– معهد بحوث الأراضي والمياه والبيئة– مركز البحوث الزراعية.

أجريت تجربتان حقليتان في محافظة المنوفية في أحدي المزارع العضوية خلال موسمي ٢٠٠٥/٢٠٠٦ و٢٠٠٦/٢٠٠٧ لدراسة تأثير التفاعل بين التسميد الحيوي بمخلوط السلالات *Azotobacter chroococcum, Glomus macrocarpum and Bacillus circulans* والتسميد العضوي في وجود بكتيريا *Pseudomonas fluorescens* على نشاط بعض الإنزيمات ومحتوى التربة من العناصر المغذية ومواصفات نمو ومحصول العشب لنباتات الدمسيسة ولقد أوضحت النتائج ما يلي:

أوضحت النتائج أن أعلي نشاط لإنزيم النيتروجينيز والفوسفاتيز قد ظهر عند معاملة التربة بالجرعة الكاملة من السماد العضوي الصناعي مع تلقيحها بالسماد الحيوي في وجود عامل المقاومة الحيوية .

وقد أدي تلقيح التربة بسلالات السماد الحيوي مع استخدام السماد العضوي الصناعي إلى الحصول علي أعلي تركيز من النيتروجين النتراتي والأمونيومي في التربة.

ومن الجدير بالذكر أنه بزيادة جرعة السماد العضوي الصناعي زاد محتوي التربة من النيتروجين الأمونيومي. كما أوضحت النتائج أن تلقيح التربة بالسماد الحيوي قد زاد من محتوي التربة من الأمونيا مقارنة بالمعاملات الغير ملقحة وقد ظهر ذلك خلال جميع مراحل نمو النبات.

كما أوضحت النتائج المتحصل عليها أن أعلي تركيز من الفوسفور الميسر قد ظهر مع التربة المسمدة بالسماد العضوي الصناعي والملقحة بمخلوط سلالات السماد الحيوي.

أظهرت النتائج زيادة صفات النمو معنويا وكذلك زيادة محصول العشب لنباتات الدمسيسة بتسميد التربة بالسماد العضوي الصناعي مع تلقيحها بمخلوط سلالات السماد الحيوي مقارنة باستخدام كلٍ منهما على حده. ومن الجدير بالذكر أنه قد تحسنت صفات النمو ومحصول العشب لنباتات الدمسيسة بزيادة جرعة السماد العضوي الصناعي وأن أعلي معدل لصفات النمو قد ظهر عند تلقيح التربة بالسماد الحيوي مع استخدام الجرعة الكاملة من السماد العضوي الصناعي.