

Role of *Azotobacter* in Soil Fertility and Sustainability—A Review

Abstract

In the present context, the best alternative of chemical fertilizer is necessary because of its adverse effects on the soil health. There are several alternatives available to enhance the soil fertility one of them is *Azotobacter*. It is a free-living N_2 -fixer diazotroph that has several beneficial effects on the crop growth and yield. It helps in synthesis of growth regulating substances like auxins, cytokinin and Gibberellic Acid (GA). In addition, it stimulates rhizospheric microbes, protects the plants from phyto-pathogens, improves nutrient uptake and ultimately boost up biological nitrogen fixation. The abundance of these bacteria in soil is related to many factors, mostly soil pH and fertility.

Keywords: *Azotobacter*; Biofertilizers; Biological nitrogen fixation; Soil sustainability

Review Article

Volume 2 Issue 6 - 2015

Arjun Dev Jnawali¹, Roshan Babu Ojha^{2*} and Sushma Marahatta¹

¹Institute of Agriculture and Animal Science, Tribhuvan University, Nepal

²Department of soil science, Purwanchal University, Nepal

***Corresponding author:** Roshan BO, Assistant Professor, Department of soil science, Himalayan College of Agriculture science and Technology, Purwanchal University, Kathmandu, Nepal, Tel: +977-9845328915; Email: roshanbachhan@gmail.com

Received: May 7, 2015 | **Published:** October 9, 2015

Introduction

Azotobacter spp. are Gram negative, free-living, aerobic soil dwelling [1], oval or spherical bacteria that form thick-walled cysts (means of asexual reproduction under favorable condition) [2]. There are around six species in the genus *Azotobacter* [3] some of which are motile by means of peritrichous flagella, others are not. They are typically polymorphic and their size ranges from 2-10 μ m long and 1-2 μ m wide [2]. The *Azotobacter* genus was discovered in 1901 by Dutch microbiologist and botanist Beijerinck et al. [4] (founder of environmental microbiology). *A. chroococcum* is the first aerobic free-living nitrogen fixer [4].

These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of *Azotobacter* cells thereby contributing towards the nitrogen availability of the crop plants. *Azotobacter* spp. is sensitive to acidic pH, high salts, and temperature [5]. *Azotobacter* has beneficial effects on crop growth and yield through, biosynthesis of biologically active substances, stimulation of rhizospheric microbes, producing phytopathogenic inhibitors [6-7]. Modification of nutrient uptake and ultimately boosting biological nitrogen fixation [8].

Besides being quite expensive and making high cost of production, chemical fertilizers have adverse effect on soil health and microbial population. In such situation, biofertilizer can be the best alternative for enhancing soil fertility. Being economic and environmental friendly, biofertilizers can be used in crop production for better yield [9]. Similarly, microbial products are considered safer, self-replicating, target specific, which is regarded as major component of integrated nutrient management from soil sustainability perspective.

Role of *Azotobacter* in soil fertility

***Azotobacter* in soil:** The presence of *Azotobacter* sp. in soils has beneficial effects on plants, but the abundance of these bacteria is related to many factors, soil physico-chemical (e.g. organic matter, pH, temperature, soil moisture) and microbiological properties

[10]. Its abundance varies as per the depth of the soil profile [11-15]. *Azotobacteria* are much more abundant in the rhizosphere of plants than in the surrounding soil and that this abundance depends on the crop species [16,17].

Nitrogen fixation: Nitrogen is the component of protein and nucleic acids and chlorophyll. Thus, nitrogen supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Therefore, adequate supply of nitrogen is necessary to achieve high yield potential in crop.

The atmosphere comprises of ~78% nitrogen as an inert, in unavailable form. Above every hectare of ground there are ~80000 tones of this unavailable nitrogen. In order to be converted to available form it needs to be fixed through either the industrial process or through Biological Nitrogen Fixation (BNF). Without these nitrogen-fixers, life on this planet may be difficult.

Nitrogen (N) deficiency is frequently a major limiting factor for crops production [18]. Nitrogen is an essential plant nutrient, widely applied as N-fertilizer to improve yield of agriculturally important crops. An interesting alternative to avoid or reduce the use of N-fertilizers could be the exploitation of Plant Growth-Promoting Bacteria (PGPB) capable of enhancing growth and yield of many plant species, several of agronomic and ecological significance.

Azotobacter spp. are non-symbiotic heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year [10]. Bacterization helps to improve plant growth and to increase soil nitrogen through nitrogen fixation by utilizing carbon for its metabolism [19].

Seed inoculation *Azotobacter* and nutrient uptake

Seed Inoculated with *Azotobacter* helps in uptake of N, P along with micronutrients like Fe and Zn, in wheat, these strains can potentially be used to improve wheat nutrition [20]. Seed inoculation of *Azotobacter* profoundly contribute to increase yield by supplying nitrogen to the crops. Inoculation of seeds with *Azotobacter chroococcum* increased carbohydrate and protein

content of two corn varieties (Inra210 and Inra260) in greenhouse experiment [21]. There is increment in Maize biomass with the application of manure and *Azotobacter* [22].

In nitrogen-deficient sand, seed inoculation increased plant length, dry weight, and nitrogen content in addition to a significant increase in soil nitrogen [23]. It was found that *A. chroococcum* at concentration of 108 cfu ml⁻¹ increased seed germination of Cucumber [2]. Seeds of wheat (*Triticum aestivum*) were inoculated with 11 bacterial strains of *A. chroococcum*, Research result showed that all *A. chroococcum* strains had positive effect on the yield and N concentrations of wheat [24].

Role of *Azotobacter* in growth substances production and promotion

Besides, nitrogen fixation, *Azotobacter* produces, Thiomin, Riboflavin, Nicotin, Indol Acetic Acid and Gibberalin. When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent. Brakel & Hilger [25] showed that *Azotobacter* produced Indol-3-Acetic Acid (IAA) when tryptophan was added to the medium. Hennequin & Blachere [26] found only small amounts of IAA in old cultures of *Azotobacter* to which no tryptophan was added.

Bacteria of the genus *Azotobacter* synthesize auxins, cytokinins, and GA-like substances, and these growth materials are the primary substance controlling the enhanced growth of tomato [27]. These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of the closely associated higher plants. Eklund et al. [28] demonstrated that the presence of *Azotobacter chroococcum* in the rhizosphere of tomato and cucumber is correlated with increased germination and growth of seedlings. Puertas & Gonzales [29] report that dry weight of tomato plants inoculated with *Azotobacter chroococcum* and grown in phosphate-deficient soil was significantly greater than that of non inoculated plants. Phytohormones (auxin, cytokinin, gibberellin) can stimulate root development.

High Gibberillic acid production was detected in *Azotobacter* (71.42 %) isolates. Higher phosphate solubilization was detected in the isolates of *Azotobacter* (74.28 %) followed by *Pseudomonas* (63.00 %) [30]. Gibberellins applied in small quantities to the soil or rosettes on the leaves and shafts of certain plant produces an increase in height. In grains such as wheat and corn, they also cause an increase in length of the leaves. In some cases they also increase the fresh weight and dry weight. However it doesn't produce any effect on the growth of the roots [31].

Brown & Burlingham [32] have found that after treating tomato seeds or seedling roots with small amounts (0.5-0.01 pg.) of commercially produced gibberellins GA 3, the plants responded in the same way as after treatments with 14-day cultures of *Azotobacter chroococcum* strain A 6. *Azotobacter chroococcum* strains isolated from the sugar beet rhizosphere were also shown to produce gibberellins; the growth of pea hypocotyl was equivalent to a GA3 concentration of 0.003-0.1 µg/cm³ culture [33]. Similarly, Cytokinins are related to nucleic acids with precursor substances that act to stimulate cell division in vegetative growth areas.

These responses suggest that *Azotobacter* probably influences the development of plants by producing growth-regulating substances. Therefore, *Azotobacter* spp. is often regarded as a

member of "Plant Growth Promoting Rhizobacteria (PGPR)".

It was also concluded that *Azotobacter* inoculants have a significant promoting effect on growth parameters like root, shoot length and dry mass of bamboo and maize seedlings in vitro and in pot experiments [2]. The dual inoculation of *A. chroococcum* and *P. indica* had beneficiary response on shoot length, root length, fresh shoot and root weight, dry shoot and root weight, and panicle number that affect growth of rice plant [34].

Adathoda vasica plants inoculated with *A. chroococcum* revealed significantly increased root nitrogen content compared to the control plants [35]. The *Azotobacters* which were inoculated with *Rhizophora* seedlings, increased significantly the average root biomass up to by 98.2%, the root length by 48.45%, the leaf area by 277.86%, the shoot biomass by 29.49% as compared to controls and they also increased the levels of total chlorophylls and carotenoids up to by 151.0% and 158.73%, respectively [36]. The *Azotobacter* inoculated crop show better growth rate. During rosette stage of canola the Crop Growth Rate (CGR) found highest i.e. 10-12 % increment [37].

Dry matter accumulation

There is increment in dry matter accumulation in *Azotobacter* inoculated plants; it stimulates development of foliage, roots, branching, flowering and fruiting which is triggered by fixed nitrogen and plant growth regulator like substance produced. It also increases plant tolerance to lack of water under adverse condition [38]. Similar result put forwarded by Sandeep et al. [39] which revealed that there is better growth response of *Azotobacter* inoculated plants as compared to non-inoculated control plants. Better crop growth response ultimately results in better dry matter accumulation.

Leaf Area Index (LAI)

Azotobacter in balanced nutrient condition results in 3.5 % increment in LAI at rosette stage of canola crop and additional application of *Azotobacter* shot up the yield by 21.17 % over the control (chemical fertilizers) [37]. The rate of increase in the leaf area determines the photosynthetic capacity of plant, which leads to better assimilation of produce and towards yield. Using *Azotobacter* spp. potato yield has been increased by 33.3% and 38.3% [38]. Similarly 20% yield increment is recorded in *Azotobacter* inoculated plants [40-41] reported that, number of branches, pod per plant and 1000 grain weight also increased with *Azotobacter* application.

Biochemical effects

Several strains of *Azotobacter* are capable of producing amino acids when grown in culture media amended with different carbon and nitrogen sources [42]. Substance like amino acid produced by these rhizobacteria are involved in many processes that explain plant-grown promotion. Biochemical analysis of chlorophyll, nitrogen, phosphorous, potassium and protein content was higher in *Azotobacter* inoculated plants as compared to non-inoculated control plants [43].

Anti-pathogenic response

Azotobacter spp. are capable to produce siderophore, they bind to the available form of iron Fe⁺³ in the rhizosphere, thus making it

unavailable to the phytopathogens and protecting the plant health; similarly Hydro Cyanine (HCN) production was higher in traits of *Azotobacter* (77.00 %) [30]. *Azotobacter* secretes an antibiotic with a structure similar to anisomycin, which is a documented fungicidal antibiotic. *Azotobacter*, in sufficient numbers, will out-compete pathogens for food. Some of the pathogens that have been controlled by *Azotobacter* in the soil and on the leaf include: *Alternaria*, *Fusarium*, *Collectotrichum*, *Rhizoctonia*, *Microfomina*, *Diplodia*, *Batryiodiplodia*, *Cephalosporium*, *Curvularia*, *Helminthosporium* and *Aspergillus*.

Effects of chemical fertilizer in *Azotobacter*

Combined application of bio-fertilizer with 50% of chemical fertilizers (N and P) has significant effect in plant growth, plant height, number of branches, fresh and dry weight of safflower in comparison with chemical fertilizers alone. Similarly, application of *Azotobacter* biophosphate and organic fertilizers, with half dose of chemical fertilizers increase the economic yield of safflower [44]. Efficiency of *Azotobacter* found decreased with increased N level [45]. The best combination was recorded with NH₄Cl at 0.1 g/L whereas, action of copper in *Azotobacter* found toxic even in very low concentration [46]. The population of *Azotobacter* may suffer due to high amount of nitrates and the acidic environment created because of chemical fertilizer.

Effect of pesticides in *Azotobacter*

Balajee & Mahadevan [47] reported that, the effect of herbicide 2, 4-D and its products; p-chlorophenoxy-acetic acid and p-chlorophenol were utilized by *A. croococcum* as carbon source, which ultimately stimulate nitrogenase enzyme. Similar result found by Kanungo et al. [48] which revealed insecticide carbofuran also stimulates nitrogenase enzyme activity. Martinez et al. [49] found that, herbicide simazine have no effect on *A. chroococcum* growth either on standard medium or on dialysed soil and sterilized soil medium. The presence of 50-300 mg of simazine per ml of culture or in one gram of soil did have a stimulating effect on *Azotobacter*. When *Azotobacter* is grown in presence of simazine, the cells have a higher ATP content than the control. Whereas organophosphorous insecticides profenofos and chloropyrifos had adverse effect on the number of aerobic nitrogen fixers and decreased nitrogen fixation [50].

Azotobacter in nutrient cycling

Azotobacter makes availability of certain nutrients like Carbon, Nitrogen, Phosphorus and Sulphur through accelerating the mineralization of organic residues in soil and avoid uptake of heavy metals [51]. *Azotobacter* can be an important alternative of chemical fertilizer because it provides nitrogen in the form of ammonia, nitrate and amino acids without situation of over dosage, which might be one of the possible alternatives of inorganic nitrogen source (eg. Urea). It also helps to sustain the plant growth and yield even in case of low phosphate content soil, as well as helps in uptake of macro and certain micro nutrients which facilitates better utilization of plant root exudates itself.

Conclusion

Azotobacter spp. are free living, non-symbiotic, heterotrophic bacteria capable of fixing an average of 20 kg N/haper year. These

bacteria are regarded as Plant Growth Promoting Rhizobacteria (PGPR) which synthesize growth substance that enhances plant growth and development and inhibit phytopathogenic growth by secreting inhibitors. It also helps in nutrient uptake and produces some biochemical substances such as protein, amino acids etc. *Azotobacter* improves seed germination and has beneficiary response on Crop Growth Rate (CGR). It helps to increase nutrient availability and to restore soil fertility for better crop response. It is an important component of integrated nutrient management system due to its significant role in soil sustainability. More research is necessary in future to explore the potentiality of *Azotobacter* in soil fertility.

Acknowledgement

Authors are grateful to Asst. Prof. Amit Khanal (Dept. of Horticulture, IAAS Lamjung) for the constructive comment during manuscript preparation.

References

- Gandora V, Gupta RD, Bhardwaj KKR (1998) Abundance of *Azotobacter* in great soil groups of North-West Himalayas. J Indian Soc Soil Sci 46(3): 379-383.
- Salhia B (2013) The Effect of *Azotobacter chroococcumas* Nitrogen biofertilizer on the growth and yield of *Cucumis sativus*. The Islamic University Gaza, Deanery of Higher Education Faculty of Science, Master of Biological Sciences, Botany.
- Martyniuk S, Martyniuk M (2003) Occurrence of *Azotobacter* spp. in some polish soils. Polish J Environ Stud 12(3): 371-374.
- Beijerinck MW (1901) Ueber Oligonitrophile Mikroben, Zentralblattfir Bakteriologie, Parasitenkunde, Infektionskrankheiten and Hygiene, Abteilung II 7: 561-582.
- Tchan YT, New PB (1989) *Azotobacteraceae*. In: Holt JG, Williams & Wilkins (Eds.) Bergey's Manual of Systematic Bacteriology Volume 1. Baltimore, USA, pp. 220-229.
- Chen J (2006) The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use, Thailand, p. 1-10.
- Lenart A (2012) Occurance Characteristics and Genetic Diversity of *Azotobacter chroococcum* in Various Soils of Southern Poland. Pol J Environ Stud 21(2): 415-424.
- Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signaling: A love parade beneath our feet. Crit Rev Microbiol 30(4): 205-240.
- Nagananda GS, Das A, Bhattacharya S, Kalpana T (2010) In vitro studies on the Effects of Biofertilizers (*Azotobacter* and *Rhizobium*) on Seed Germination and Development of *Trigonella foenum-graecum* L. using a Novel Glass Marble containing Liquid Medium. International Journal of Botany 6 (4): 394-403.
- Kizilkaya R (2009) Nitrogen fixation capacity of *Azotobacter* spp. Strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J Environ Biol 30(1): 73-82.
- Vojinovic Z (1961) Microbiological properties of main types soil in Serbia for nitrogen cycling. Journal for Scientific Agricultural Research 43: 3-25.

12. Sariv Z (1969a) Biogenic levels of the horizons of calcereous chernozem in Vojvodina. *Contemporary Agriculture* 17: 819-825.
13. Sariv Z (1969b) Biogenity of limeless chernozem in Vojvodina. *Annals of Scientific Work at the Institute for Research in Agriculture Novi Sad* 7: 145-151.
14. Malek AY, Hosny I, Shawky BT (1979) Nitrogen-fixing capacity of *Azotobacter* as affected by the type and depth of substrate. *Zentralbl Bakteriell Naturwiss* 134(5): 390-397.
15. Kalaigandhi V, Kannapiran E, Harimuraleedharan, Michael A, Sivakumar T, et al. (2010) *Azotobacter* population in Rhizosphere and Non-Rhizosphere sediments of Tondi Coast. *International Journal of Biological Technology* 1(1): 63-65.
16. Sariv Z, Ragoviv B (1963a) The influence of the maize on the dynamic of *Azotobacter* in the soil. *Soil Plant* 13: 273-277.
17. Sariv Z, Ragoviv B (1963b) The effect of some plants on the dynamics of *Azotobacter* in the soil. *Annals of Scientific Work at the Faculty of Agriculture Novi Sad* 7: 1-11.
18. Dong H, Li W, Eneji AE, Zhang D (2012) Nitrogen rate and plant density effects on yield and late-season leaf senescence of cotton raised on a saline field. *Field Crops Research* 126: 137-144.
19. Monib M, Hosny I, Fayez M (1979a) Effect of *Azotobacter* inoculation on plant growth and soil nitrogen. *Zentralbl Bakteriell Naturwiss* 134(2):140-148.
20. Rajae S, Alikhani HA, Raiesi F (2007) Effect of Plant Growth Promoting Potentials of *Azotobacter chroococcum* Native Strains on Growth, Yield and Uptake of Nutrients in Wheat [2007-10]. *Agris records* 11(41): 285-297
21. Kizilog IU, Bilen FT, Ataplu N (2001) Effect of Inoculation Eight *Azotobacter chroococcum* and Nitrogen Fertilizer on Plant Growth of Corn (*Zea mays*) Carbohydrate and Protein Contents. *Ziraat Fakultesi Dergisi Ataturk Universitesi* 32: 215-221.
22. Meshram SU, Shende ST (1982) Response of Maize to *Azotobacter chroococcum*. *Plant and Soil* 69: 265-273.
23. Monib M, Malek AEY, Hosny I, Fayez M (1979b) Seed inoculation with *Azotobacter chroococcum* in sand cultures and its effect on nitrogen balance. *Zentralbl Bakteriell Naturwiss* 134(3): 243-248.
24. Kizilkaya R (2008) Yield response and nitrogen concentrations of spring wheat (*Triticum aestivum*) inoculated with *Azotobacter chroococcum* strains. *Ecological Engineering* 33(2): 150-156.
25. Brakel J, Hilger F (1965) Etude qualitative et quantitative de la synthese de substances de nature auxinique par *Azotobacter chroococcum* in vitro. *Bull Inst Agron Stns Rech Gembloux* 33: 469-487.
26. Hennequin JR, Blachere H (1966) Recherches sur la synthese de phytohormones et de composes phenoliques par *Azotobacter* et des bacteries de la rhizosphere. *Ann Inst Pasteur* 3: 89-102.
27. Azcorn R, Barea JM (1975) Synthesis of auxins, gibberellins and cytokinins by *Azotobacter vinelandi* and *Azotobacter beijerinckii* related to effects produced on tomato plants. *Plant Soil* 43: 609-619.
28. Eklund E (1970) Secondary effects of some *Pseudomonads* in the rhizosphere of peat grown cucumber plant. In: Pharis RP & Reid DM (Eds.), *Hormonal Regulation of Development* 3: p. 613.
29. Puertas A, Gonzales LM (1999) Aislamiento de cepas nativas de *Azotobacter chroococcum* en la provincial Granmayevaluacion de su actividad estimuladora en plantulas de tomate. *Cell Mol Life Sci* 20: 5-7.
30. Althaf HS, Srinivas P (2013) Evaluation of Plant Growth Promoting Traits by *Pseudomonas* and *Azotobacter* Isolated From Rhizotic Soils of Two Selected Agro forestry Tree Species of Godavari Belt Region, India. *Asian J Exp Biol Sci* 4 (3): 431-436.
31. BEN J (1964) *Tratado de Botánica Edic*, Omega SA, Barcelona, España, p.747.
32. Brown ME, Burlingham S K (1968) Production of Plant Growth Substances by *Azotobacter chroococcum*. *J gen Microbiol* 53: 135-144.
33. Mrkovic N, Milic V (2001) Use of *Azotobacter chroococcum* potentially useful in agricultural application *Annals of Microbiology* 51: 145-158.
34. Kamil P, Yami KD, Singh A (2008) Plant Growth Promotional Effect of *Azotobacter chroococcum*, *Piriformospora indica* and Vermicompost on Rice Plant. *Nepal Journal of Science and Technology* 9: 85-90.
35. Anantha NT, Earanna N, Suresh CK (2007) Influence of *Azotobacter chroococcum* strains on growth and biomass of *Adathoda vasica* Nees. *Karnataka J Agric Sci* 20 (3): 613-615.
36. Ravikumara S, Kathiresanb K, Thadedus M, Selvama BM, Shanthya S, et al. (2004) Nitrogen-fixing *Azotobacters* from mangrove habitat and their utility as marine biofertilizers. *Journal of Experimental Marine Biology and Ecology* 312(1): 5-17.
37. Yasari E, Patwardhan AM (2007) Effect of (*Azotobacter* and *Azospirillum*) Inoculants and Chemical Fertilizers on Growth and Productivity of Canola (*Brassica napus* L.). *Asian Journal of Plant Sciences* 6(1): 77-82.
38. Zeña GG, Peru C (1986) Effect of Different Rates of *Azotobacter* and Frequency of Application of Agrispon on Yield and Quality in the Growing of Onion (*Allium cepa* L.) in Cajamarca. *National University of Cajamarca Faculty of Agriculture Sciences and Forestry*.
39. Sandeep C, Rashmi SN, Sharmila V, Surekha R, Tejaswini R, et al. (2011) Growth Response of *Amaranthus gangeticus* to *Azotobacter chroococcum* Isolated from Different Agroclimatic Zones of Karnataka. *Journal of Phytology* 3(7): 29-34.
40. BLACK C (1975) *Relaciones Suelo-Planta*. Tomo II (Ed.), Hemisferio Sur. 1° Edic. Bs. As. P. 866
41. Estiyar HK, Khoei FR, Behrouzfar EK (2014) The effect of nitrogen biofertilizer on yield and yield components of white bean (*Phaseolus vulgaris* cv. Dorsa). *International Journal of Biosciences* 4(11): 217-222
42. López GJ, Pozo RB, López S, Toledo M, Salmerón V, et al. (2005) Liberation of amino acids by heterotrophic nitrogen fixing bacteria. *Amino Acid* 28(4): 363-367.
43. Naseri R, Azadi S, Rahimi MJ, Maleki A, Mirzaei A (2013) Effects of Inoculation with *Azotobacter chroococcum* and *Pseudomonas putida* on yield and some of the important agronomic traits in Barley (*Hordeum vulgare* L.). *International Journal of Agronomy and Plant Production* 4(7): 1602-1610.
44. Ojaghloo F, Farahvash F, Hassanzadeh A, Pouryusef M (2007) Effect of inoculation with *Azotobacter* and barvar phosphate bio-fertilizers on yield of safflower (*Carthamus tinctorius* L.). *Journal of Agricultural Sciences, Islamic Azad University, Tabriz Branch*, p. 25-30.
45. Soleimanzadeh H, Gooshchi F (2013) Effects of *Azotobacter* and Nitrogen Chemical Fertilizer on Yield and Yield Components of Wheat (*Triticum aestivum* L.). *World Applied Sciences Journal* 21 (8): 1176-1180
46. Gül FS (2003) Growth and nitrogen fixation dynamics of *Azotobacter chroococcum* in nitrogen-free and OMW containing medium. *The middle east technical university* 1-12.

47. Balajee S, Mahadevan A (1990) Influence of chloroaromatic substances on the biological activity of *Azotobacter chroococcum*. Chemosphere 21 (1-2): 51-56.
48. Kanungo PK, Adhya TK, Rao V R (1995) Influence of repeated applications of carbofuran on nitrogenase activity and nitrogen - fixing bacteria associated with rhizosphere of tropical rice. Chemosphere 31 (5): 3249-3257.
49. Martinez T, Salmeron M V, Gonzalez L (1991) Effect of simazine on the biological activity of *Azotobacter chroococcum*. Soil Science, 151 (6): 459-467.
50. Martinez T, Salmeron MV, Gonzalez L (1992) Effects of an organophosphorus insecticide, profenofos on agricultural soil microflora. Chemosphere 24 (1): 71-80.
51. Lévai L, Szilvia V, Nóra B, Éva G (2008) Can wood ash and biofertilizer play a role in organic agriculture? Agronomski Glasnik 3: 263-271