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Effect of *Azotobacter* and PSB Inoculation on Rhizosphere of Tomato

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ABSTRACT: A study was conducted in the Department of Agricultural Microbiology, IGKV during the year 2021-22 to assess the inoculation effect on soil biology in the rhizosphere region of tomato. Studies on the complex microbes–rhizosphere environment and the evaluation of plant growth promotion are considered necessary. Revealing out the details of characterization of *Azotobacter* and PSB isolates were carried out and their effect on plant growth promotion were more focused as sole and dual inoculation. The study was conducted with tomato crop in Completely Randomized Design with three replicates and eight treatments. Inoculation treatments consisted of sole applications of two strains of *Azotobacter* (Azoto-B-26 and Azoto-B-24) and two strains of PSB (PSB-S-91 and PSB-S-68) and their dual inoculations. Considering the ill-effects of the inorganic fertilizers and the positive aspects of the microbial inoculants, the present investigation was undertaken to assess the dual inoculation effect of *Azotobacter* isolate in combination with PSB on the rhizosphere of tomato. The cultural and biochemical characteristics of isolates were studied, which showed positive effect with tests of starch hydrolysis test, catalase test and urease test, while as per the oxidase test except Azoto-B-24 all showed positive reaction. The experimental results revealed that significantly maximum plant height (86.8 cm), plant shoot dry biomass (23.3g plant^{-1}), plant root dry biomass (8.56 plant^{-1}) were noted under T_7 (Azoto-B-24 + PSB-S-91) followed by T_8 (Azoto-B-24 + PSB-S-68). Better microbial population (*Azotobacter* and PSB population) in tomato rhizosphere soils was observed under the treatments T_7 and T_8. Similarly Dehydrogenase activity was recorded maximum as 26.3 µgTPF/g soil/ hour under treatment T_7. On the basis of the experimental findings, dual inoculation of promising strains of *Azotobacter* (Azoto-B-26) and PSB (PSB-S-91) through root inoculation treatment gave better soil biological properties in tomato.

Keywords: *Azotobacter,* PSB, Inoculation, Tomato, Rhizosphere and Biomass.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important, popular and widely grown vegetable in India. It belongs to family Solanaceae and is believed to be a native of western South America. This crop is also known as an industrial crop because of its outstanding processing qualities. Tomato is rich source of minerals, vitamins and organic acid such as citric, malic, and acetic acid which are known as healthy acids in fresh tomato fruit and tomato fruit provides 3-4% total sugar, 4-7% total solids, 15-30 mg/100g ascorbic acid and 20-50 mg/100g fruit weight of lycopene (Giovannucci, 1999). It is one of the most popular salad vegetables and taken with great relish. It is widely employed in cannery and made into soups, pickles, ketchup, sauces, juice etc (Kumar *et al*., 2019). Several epidemiological studies indicated beneficial effects of tomato consumption in the prevention of some major chronic disease, such as cancer and cardiovascular disease (Giovannucci, 1999). Phosphorus being the limiting nutrient available mostly in immobile form in soil system. Phosphorus solubilizing bacteria readily makes this nutrient available to the plant, by involving several mechanisms which reduces the soil pH for making the insoluble form of phosphorus available to plants in soluble form (Bagyaraj *et al*., 2000). The average yield of tomato in India is not according to the crop potential, for which many factors are responsible i.e., intensive chemical treatments, inadequate use of fertilizers, crop variety etc. The crop particularly hybrids have higher requirements of nutrients. Low use of fertilizers and imbalance in NPK application ratio is partially responsible for the low yield (Kamal *et al.,* 2018). In addition, complete dependence on chemical fertilizers is not able to produce the higher yields with soil quality sustainability. Also, inorganic fertilizers costs higher and the use of bioinoculants along with chemical fertilizers are of need (Ritu and Dash 2022).

Azotobacter is a heterotrophic free living nitrogen fixing bacteria present in alkaline and neutral soils. *Azotobacter chrococcum* is the most commonly occurring species in arable soils of India. Apart from its ability to fix atmospheric nitrogen in soils, it can also synthesize growth promoting substances *viz.,* auxins and gibberellins and also to some extent the vitamins. Among the biofertilizers, *Azotobacter* as nitrogen fixer

and PSB as phosphate solublizer have gained much importance, and there has been encouraging response of crops to inoculation with *Azotobacter* and PSB. These non conventional sources of fertilizer are not only cost effective but simultaneously boost up the productivity of soil and crop to a large extent. Experiments carried out on *Azotobacter* indicated that this is suitable when inoculated in vegetable crops like onion, brinjal, tomato and cabbage. Seed treatment with bacteria as a biofertilizer effective in boosting and increasing the growth performances of tomato plants (Nurlila *et al.,* 2020). Previously it has been studied that application of *Azotobacter* and *Azospirillum* gave better plant growth and fruit yield (Kamal *et al.,* 2018). An attempt was made to develop suitable bio-inoculants for tomato growers of Chhattisgarh. Keeping these in view, response of inoculation of *Azotobacter* and PSB on growth of tomato and the soil microbial properties as affected by inoculation were studied.

MATERIAL AND METHODS

Two strains each of *Azotobacter (*Azoto-B-26 and Azoto-B-24) and PSB (PSB-S-91 and PSB-S-68) used in this experiment were collected from Departmental Culture Collection Bank, Department of Agricultural Microbiology, IGKV, Raipur. The strains of *Azotobacter* and PSB were revived by inoculating on Jensen's media and Pikovskaya's Agar Medium at pH 7.0 and temperature 28±2°C respectively. After 24-48 hours of incubation, bacterial colonies were observed for colony morphological characteristics. The morphological characteristics of bacterial colonies were determined according to the elevation, color, size and margin of the discrete colonies. The phenotypical characters and growth pattern of the isolates were observed under microscope and recorded. Gram staining of *Azotobacter* and PSB was carried out as per Aneja (2003).

Following biochemical characteristics were carried out as follows

Starch Hydrolysis test: Loop-full of each culture was inoculated on Starch Agar plates by streaking. The slants were incubated at 28±2°C and after 24 hours, the plates were flooded with iodine solution. Distinct clear zone of inhibition around the bacterial colonies indicating the starch hydrolyzing potential.

Catalase Test: A small quantity of bacterial culture is transferred onto a dry sterilized glass slide by using inoculating loop and a drop of H_2O_2 is placed on the bacterial sample to observe the formation of bubbles i.e. the effervescence of oxygen gas (within 5-10 sec) depicts a positive for catalase test as reported by Akhter *et al.* (2012).

Urease Test: Urease agar slants were inoculated with the *Azotobacter* and PSB culture and were incubated at 37ºC for 24 hours. Urease hydrolysis activity is confirmed if the bacterial growth region turns pink.

Oxidase test: A fresh culture (18 to 24) hours of bacteria was grown on nutrient agar using the streak plate method so that well isolated colonies are present, A small piece of filter paper soaked in 1% Kovacs oxidase reagent and after air dry, kept on cultured bacterial plate. Colour changes to dark purple within 5-10 seconds are Oxidase positive and colour does not change or take longer than 2 minutes are negative in oxidase test.

Crop growth parameters: Tomato crops were grown by inoculating with *Azotobacter* and PSB.

At the time of initiation of flowering, rhizosphere soil samples were taken for microbial properties analysis in laboratory. Plant growth parameters of plant height and biomass accumulation were recorded.

Soil biological properties: Dehydrogenase activity of soil is measured by the absorbance of the sample solution treated with Triphenyl Tetrazolium Chloride (Wolinska and Stępniewska 2012). Estimation of Microbial population in rhizosphere soil samples was carried out by serial dilution and plating method (Subba Rao, 2000).

Number of microbes per gram of oven dry soil =

$$\frac{\text{No. of colony forming units (cfu)} \times \text{dilution}}{\text{Dry weight of one g moist soil sample} \times \text{aliquot taken}}$$

The data on numerous parameters were statistically analyzed, as proposed by Panse and Sukhatme (1967) to determine the impact of various treatments effect.

RESULTS AND DISCUSSION

Seed inoculation with bacteria as bioinoculants was effective in boosting and increasing the growth performances of tomato plants. *Azotobacter* and PSB isolates required for inoculation were revived and their morphological and biochemical tests were carried out. *Azotobacter* isolates showed colonies of gel like translucent, round and convex in shape, colony have entire margin. Colonies of PSB isolate were found to be round, pale white in color in Pikovskaya's medium and had a clearing zone surrounding colony was observed. Both the isolates were found to show gram negative in response to the gram's staining test. The biochemical characterization of *Azotobacter* and PSB isolates, displayed positive reaction for starch hydrolysis test, catalase test and urease test, while as per the oxidase test except Azoto-B-24 all showed positive reaction. These findings were in close agreement with Singh and Jha (2015). The results were depicted in Table 1 and Plate 1.

Plant height and biomass accumulation of tomat. Plant height significantly increased maximum to 77.83 cm under the treatment T_7 followed by the treatment T_8 with the height of 76.23. Treatments comprising dual inoculation increased the plant height significantly and are depicted in Fig. 1. The result were in agreement with Gajbhiye *et al.* (2003). As reported by Sandhu and Gill (2011), treatment of *Azotobacter* inoculation + 75% NPK + FYM gave maximum leaf area and minimum days to fruit picking. Biological routes in enhancing soil health for maximizing growth performances is a vital component of integrated nutrient management.

The maximum shoot dry weight at 45 DAT was also seen in the treatment T_7 (Azoto-2 + PSB-1) with 23.3 g per plant as compared to T_5 (Azoto-1 + PSB-2) with 18.06 g per plant. That was followed by T_2 (Azoto-2)

with a dry shoot weight of 17.26 g per plant. The lowest shoot dry weight was seen in the treatment T_4 (PSB-2) with a weight of 15.23 g per plant. Likewise root biomass at 45 DAT was significantly maximum under the treatment T_7 (Azoto-2 + PSB-1) with 8.56 g per plant as compared to T_5 (Azoto-1 + PSB-2) with 6.33 g per plant. The lowest root dry weight was seen in the treatment T_4 (PSB-2 alone) with a weight of 5.2 g per plant. These results obtained from current study are depicted in Table 2 and were similar to the research done by Mahdi *et al.* (2011) who investigated the effect of *Azotobacter chroococum*, was more than *Pseudomonas putida* and the combined inoculation produced the higher results than the control or sole application of either inoculants. Maximum growth was obtained due to the inoculation of PSB and *Azotobacter* in combination. Similar results were obtained by Argaw (2012) who revealed that the parameters like the height of the plant were enhanced by the co-inoculation of PSB and *Azotobacter* than single inoculation. Treating roots of tomato seedlings with *Azotobacter* cultures accelerates plant growth due to secretion of growth promoting substances of auxins, gibberellin, cytokinin like substances.

It has been reported that Azotobacter inoculation increased N levels in soil. This increase in N status might be partly attributed to stimulative effect of plant bioregulators which, in turn, increased the rate of nutrient absorption and translocation within the plant system consequently, more N accumulated in the plant parts (Awasthi *et al.,* 1996) resulting in overall growth. Increased plant growth might be due to more efficient absorption of nutrient elements because of the better root system developed by biofertilization i.e., N addition through biological nitrogen fixation by Azotobacter. These results are in accordance with the findings of Anburani and Manivannan (2002) in brinjal, Kumar and Kumar (2022) in okra.

Biological properties of rhizosphere soil in tomato crop. Microbial population study in rhizosphere soil presented in Fig. 2 showed that the population density of *Azotobacter* in soil varied between 5.33×10^6 to 24.66×10^6 per g of soil as per different treatments. Under treatment T_7 Maximum *Azotobacter* population was found as 24.66×10^6 and PSB population was found as 26.33×10^6. Similar findings were also reported by El-Tantawy and Mohamed (2009). At flowering stage the dehydrogenase activity of the soil sample from the experimental site was significantly high in the rhizosphere soil. The dual inoculated treatments had shown significantly maximum in dehydrogenase activity over others treatments i.e., T_7 (26.3), T_8 (25.3), T_6 (20.6), T_5 (18.3), T_1 (16.6) µg TPF/ h /g soil and minimum in T_4 (13.3 µg TPF/ h /g soil), as depicted in Table 2. Bhagat *et al.* (2014); Wyszkowska and Kucharski (2004) also expressed the similar views. The earlier studies revealed that the enzyme activities are often used as indices of microbial growth in soil i.e., indication of soil biological health.

Improvement in these parameters might be due to the secretion of ammonia into the rhizosphere, accelerated mobility of photosynthates in the presence of root exudates, (Harikrishna *et al.*, 2002; Sengupta *et al.*, 2002). The biological activity of the microorganisms would have helped the soil status to become a ready to serve zone for essential nutrients to plant's root system. Similar results were reported in coriander (Subramanian and Vijayakumar 2001) and tomato (Renuka and Ravi Shankar 2001).

Table 1: Biochemical characterization of *Azotobacter* and PSB strains.

Biochemical test	*Azotobacter*		PSB	
	Azoto- B-26	Azoto- B-24	PSB-S-91	PSB-S-68
Starch hydrolysis	+ve	+ve	+ve	+ve
Catalase test	+ve	+ve	+ve	+ve
Urease test	+ve	+ve	+ve	+ve
Oxidase test	+ve	-ve	+ve	+ve

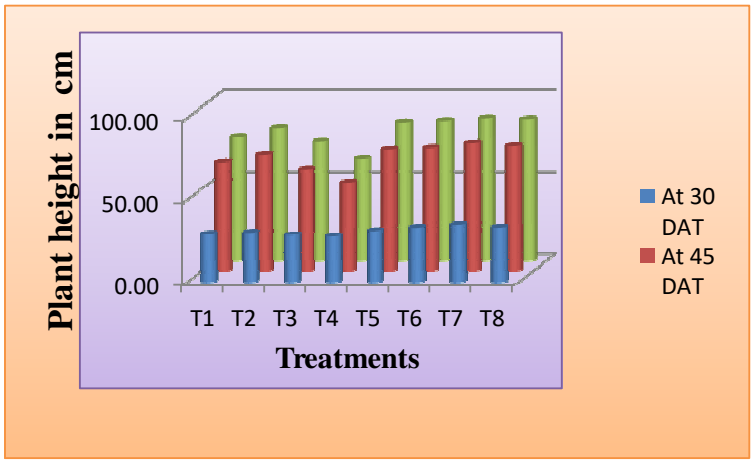
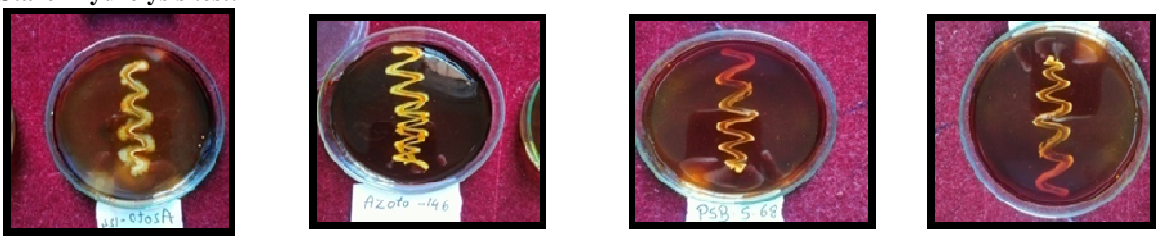


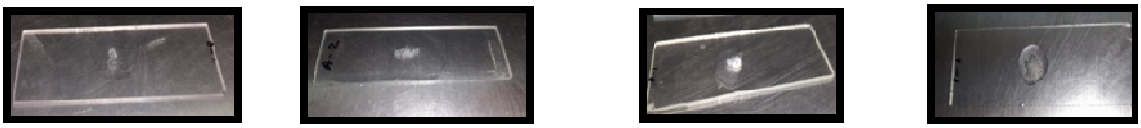
Fig. 1. Comparison of plant height at different growth stages of tomato.

Starch hydrolysis test:



Azoto-B-26 Azoto-B-24 PSB-S-91 PSB-S-68

Catalase test:

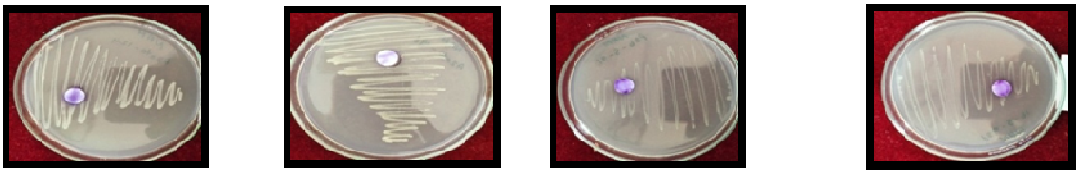


Azoto-B-26 Azoto-B-24 PSB-S-91 PSB-S-68

Urease test:

Blank AzotoB26 Azoto-B-24 PSB-S-91 PSB-S-68

Oxidase test:-



Azoto-B-26 Azoto-B-24 PSB-S-91 PSB-S-68

Plate 1: Biochemical characterization of *Azotobacter* and PSB strain.

Table 2: Effect of *Azotobacter* and PSB inoculation on Biomass accumulation of tomato and dehydrogenase activity in tomato rhizosphere soil.

Treatment	Treatment details	Dry weight(g/plant)		Dehydrogenase activity of µg TPF/ h /g soil
		Shoot	Root	
T_1	Azoto-B-26	16.13	6.21	16.6
T_2	Azoto-B-24	17.26	5.80	17.3
T_3	PSB-S-91	16.70	5.8	15.6
T_4	PSB-S-68	15.23	5.2	13.3
T_5	Azoto-B-26+ PSB-S-68	18.06	6.33	18.3
T_6	Azoto-B-26+ PSB-S-91	20.36	6.93	20.6
T_7	Azoto-B-24+ PSB-S-91	23.30	8.56	26.3
T_8	Azoto-B-24+ PSB-S-68	22.06	7.36	25.3
SEm ±		0.45	0.28	0.95
CD (P=0.05)		1.38	0.86	2.873

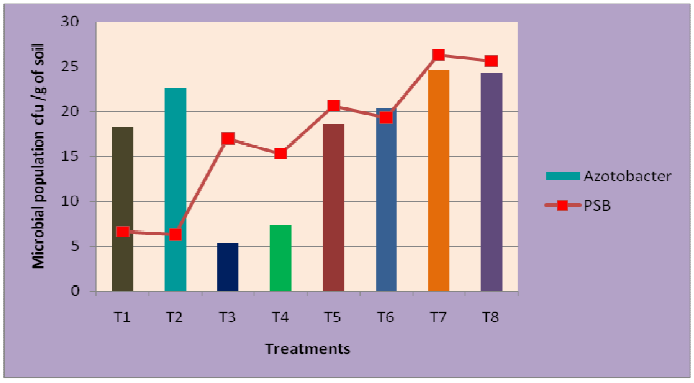


Fig. 2. Microbial population in rhizosphere soil of tomato at flowering stage.

CONCLUSIONS

From present investigation, it can be concluded that tomato crop receiving dual inoculation of *Azotobacter* (Azoto-B-24) and PSB (PSB-S-91) recorded superior plant growth, biomass and greatest microbial population in rhizosphere region of tomato plant. The treatments consisting bioinoculants recorded significantly higher values compare to without bioinoculants. Dual inoculation of *Azotobacter* (Azoto-B-24) and PSB (PSB-S-91) has proved to be the most superior treatment in respect of enhancing soil biological properties and growth performance of tomato.

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Conflicts of Interest. None.

REFERENCES

Akhter, M. S., Hossain, S. J., Hossain, A. S. K. and Datta, R. K. (2012). Isolation and characterization of salinity tolerant *Azotobacter* sp. *Greener J. Biol. Sci., 2*(3), 43-51.

Anburani, A. and Manivannan, K. (2002). Effect of integrated nutrient management on growth in brinjal. *South Indian Horticulture*, *50*(4-6), 377-386.

Aneja, K. R. (2003). Gram staining of bacteria. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International (P) Ltd. New Delhi, 102-105.

Argaw, A. (2012). Evaluation of co-inoculation of *Bradyrhizobium japonicum* and Phosphate solubilizing *Pseudomonas* spp. effect on soybean (*Glycine max* L. Merr.) in Assossa Area. *Journal of Agricultural Science and Technology*, *14*(1), 213-224.

Awasthi, R. P., Godara, R. K. and Kaith, N.S. (1996). Interaction effect of VA mycorrhizae and Azotobacter inoculation on peach seedlings. *Indian Journal of Horticulture*, *53*(1), 8-13.

Bagyaraj, D. J., U. P. Krishnaraj and S. P.S. Khanuja (2000). Mineral phosphate solubilization: agronomic implications, mechanisms and molecular genetics. *Proc. Indian Natl. Sci. Acad*., *66*(B), 69-82.

Bhagat, P. K., Dash, D., Raj, A. and Jhariya, M. K. (2014). Effect of *Rhizobium* inoculation on Growth and Biomass accumulation in *Leucaena leucocephala*. *The Ecoscan: An International Quarterly J. of Environmental Sciences*, *5*, 65-74.

El-Tantawy, M. E. and Mohamed, M. A. N. (2009). Effect of inoculation with phosphate solubilizing bacteria on the tomato rhizosphere colonization process, plant growth and yield under organic and inorganic fertilization. *Journal of Applied Sciences Research*, *5,* 1117-1131.

Gajbhiye R. P. Sharma R. R. and Tewari R. N. (2003). Effect of bio-fertilizers on growth and yield parameters of tomato. *Indian Journal of Horticulture*, *60*(4), 368-371.

Giovannucci, E. (1999). Tomato, tomato based product, lycopene, and cancer: review of the epidemiologic literature. *Journal of the National Cancer Institute*, *91*(4), 317-331.

Harikrishna, B. L., Channal, H. T., Hebsur, N. S., Dharmatti, P. R. and Sarangamath, P. A. (2002). Integrated Nutrient Management (INM) on availability of nutrient uptake and yield of tomato. *Karnataka The Journal of Agricultural Science, 15,* 275-278 .

Kamal, S., Kumar, M., Rajkumar and Raghav, M. (2018). Effect of Biofertilizers on Growth and Yield of Tomato (*Lycopersicon esculentum* Mill). *Int. J. Curr. Microbiol. App. Sci*, *7*(2), 2542-2545.

Kumar, D. and Kumar, S. (2022). Effect of Nitrogen, Organic Manures along with Biofertilizers on Yield Parameters of Okra (*Abelmoschus esculentus* L.). *Biological Forum – An International Journal*, *14*(2a), 9-12.

Kumar, Vinod, Ram, R. B., Srivastava, Abhijeet and Pandey, Raj, Hari Baksh, Singh, Himanshu and Singh, Maneesh Kumar (2019). Effect of Organic Manures and Biofertilizers on Growth, Yield and Quality Traits of Tomato (*Solanum lycopersicum* Mill.) cv. Roma. *Int. J. Curr. Microbiol. App. Sci., 8*(3), 994-999.

Mahdi, Z., Kourosh, O. and Omid, A. (2011). Effects of PGPR and AMF on growth of two bred cultivars of tomato. *Advanced environmental biology, 5*(8), 2177-2181.

Nurlila, R. U., Bahrun, A., Sutariati, G. A. K. and La Fua, J. (2020). Effect of indigenous biofertilizer treatment as growth promote on the vegetative growth of tomato. *International Journal of scientific & Technology research*, *9*(2), 1015-1018.

Panse, V. G. and Sukhatme, P. V. (1967). Statistical methods of agricultural workers. Ed. 2nd, ICAR, New Delhi, India.

Renuka, B. and Ravi Shankar, C. (2001). Effect of organic manures on growth and yield of tomato. *South Indian Horticulture, 49*(Special), 216-217.

Ritu and Dash, Diptimayee (2022). Effect of Biofertilizer and Foliar Spraying of Zinc on Growth Performance in Cucumber (*Cucumis sativus* L.). *Frontiers in Crop Improvement*, *10*(5), 2564-2568.

Sandhu, S. and Gill, B. S. (2011). Effect of integrated nutrient management strategies on growth and yield of Cape gooseberry (*Physalis peruviana* L.). *Journal of Horticultural Sciences*, *6*(1), 29-32.

Sengupta, S. K., Dwivedi, Y. C. and Kushwah, S. S. (2002). Response of tomato to bio-inoculants at different levels of nitrogen. *Vegetable Science*, *29*, 186-188.

Singh, R.P. and Jha, P. N. (2015). Plant growth promoting potential of ACC deaminase rhizosphere bacteria isolate from *Aerva javanica*: A plant adapted to saline environments. *Int. J. Curr. Microbiol. App. Sci, 4*(7),142-152.

Subba Rao, N. S. (2000). Soil Microbiology. 4th ed. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Calcutta. PP 407.

Subramanian, S. and Vijayakumar, M. (2001). Effect of various levels of nitrogen and Azospirillum on growth and yield of CO_3 coriander. *South Indian Horticulture, 49* (Special), 191-192.

Wolinska, A. and Stępniewska, Z. (2012). Dehydrogenase activity in the soil environment. *Dehydrogenases*, pp.183-210.

Wyszkowska, J. and Kucharski, J. (2004). Biochemical and physicochemical properties of soil contaminated with herbicide Triflurotox 250 EC. *Polish Journal of Environmental Studies*, *13*(2), 223-231.

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