

## RETRIEVAL EFFECT OF *AZOTOBACTER* INGRADED DOSES IN RELATION TO GROWTH, AND BIOCHEMICAL CHARACTERISTICS ON PAPER MILL EFFLUENT TREATED *ELEUSINECORACANA*, *GAERTN*

**Selvarathi P. and Murugalakshmikumari R.**

Dean, College Development Council (CDC), Bharathiar University, Coimbatore, Tamil Nadu  
Department of Botany, V.V.Vanniaperumal College for Women, Virudhunagar, Tamil Nadu



### ABSTRACT:

Bioremediation forms evacuates the toxin and it's harmfulness through metabolic responses intervened by the microorganisms. The paper plant gushing treatment on ragi created a lofty decrease in its development, color substance and in other biochemical characteristics. On bioremediation treatment, different convergences of biofertilizer *Azotobacter* 1.5%, 2%, 2.5% and 3% (w/v) were blended independently with 30% (v/v) paper factory profluent, separately on *Eleusinecoracana*, *Gaertn*. has brought out impressive increment on the development and biochemical attributes than when it was treated with emanating alone.

### KEYWORDS:

Azotobacter, Biochemical, Paper Mill.

### INTRODUCTION

Paper mill is one of the main industries in Sivakasi. In dry place like Sivakasi, due to acute water scarcity, the untreated effluents which are highly toxic to the growth of the plant system is being used as a source of irrigation for agriculture, this very badly affected the agriculture. As this environmental problem has aroused a serious concern, a safe and low cost Bio remedial treatment has been suggested as a remedial measure.

The present investigation attempts to exploit *Azotobacter* for the remediation of paper mill effluent on the growth and biochemical profile of *Eleusinecoracana*, *Gaertn*.

### MATERIALS AND METHODS

Sound and feasible seeds of *Eleusinecoracana*, *Gaertn*., were surface cleaned with 0.1% mercuric chloride, the seeds were permitted to develop in pots containing consistently blended red, dark and sandy soil in 1:1:1 proportion. Different convergences of paper factory profluent were readied, for example, 10%, 15% 20%, 25%, and 30% (v/v). The exploratory sets were kept in diffused light at room temperature. Seven days subsequent to sowing, the exploratory plants were watered always consistently with the individual centralization of the emanating (750 mL). The control sets were kept up with faucet water. Both trial sets and

control sets were kept up in triplicates. On the twenty first day both the arrangements of plants were taken for examination.

### **BIOREMEDIATION**

Biofertilizer was acquired from Agricultural Research Station, Srivilliputhur. In the bioremediation treatment, different centralizations of biofertilizer viz. 1.5%, 2%, 2.5% and 3% (w/v) of *Azotobacter* were blended independently with 30% (v/v) paper factory profluent, since that focus was observed to be ideal for *E. coracana*.

The *E. coracana* seeds were sown to bring the seedlings up in marked pots. Control sets in triplicates were kept up with faucet water and paper factory profluent (30% (v/v)). From the seventh day onwards the seedlings of *E. coracana* were treated with 750 mL of profluent blended with biofertilizer in the separate focuses. On the 21st day the seedlings were culled out with no harm and examined for the development, color, biochemical and catalyst exercises.

### **BIOCHEMICAL CHARACTERS**

For chlorophyll and carotenoids, new leaf tissues were evacuated with 100% CH<sub>3</sub>CO and were assessed using the formula prescribed by Wellburn and Lichtenthaler (1984). Complete sugar content (Jayaraman 1981), amino destructive (Moore Stein *et al.*, 1948), dissolvable leaf protein (Lowry *et al.*, 1978), proline (Bates *et al.*), Total phenol (Bray and Thorpe, 1954), in vivo nitrate reductase (Jaworski, 1971), peroxidase (Addy and Goodman, 1972), and catalase development (Kar and Mishra, 1976) were bankrupt down both for control and exploratory seedlings.

### **RESULT AND DISCUSSION**

Development Characteristics *Azotobacter* causes mineralization of settled phosphate of soil and in this manner expands uptake of phosphate in plants. A crisply disengaged strain of *A. chroococcum* fixed nitrogen all the more effectively when developed in relationship with another dirt bacterium (Parker, 2003).

The uses of different centralizations of paper factory emanating had achieved significant decrease in the shoot length and root length, crisp weight, dry weight and leaf territory of the trial plants.

Expansion of the different convergences of *Azotobacter* with paper plant emanating brought about a critical increment on the development of the seedlings. *A. vinellandi* majorly affected the mash and paper industry emanating debasement. Among the diverse joined medicines, the best results were acquired with the photocatalytic pre-treatment (S. G. Moraes *et al.*, 2006). *Azotobacter* helps in the supply of supplements development substances, anti-infection agents and optional metabolites (SubbaRao, 1982).

Expanding pattern in the shoot length and root length was seen with different convergences of *Azotobacter* integrated with paper plant gushing. There was a slow increment in the root length with the expanding centralizations of *Azotobacter*. The expansion of *Azotobacter* resulted in essentially more noteworthy yield than that given by the prescribed measurement of N. The utilization of nitrogen altering microscopic organisms, especially the *Azotobacter*, as a foliar biofertilizer expanded mulberry leaf generation (Sudhakaret *et al.*, 2000). *Azotobacter* immunization in mix with triptophan gave most extreme length and weight of maize roots and shoots than control (Zahir Ahmad Zahiret *al.*, 2000).

The utilization of *Azotobacter* coordinated with paper plant profluent demonstrated better results in the new weight, dry weight and leaf zone than the control sets treated with paper factory gushing alone. Vegetative development of *Gladiolus* was upgraded most viably by *Azotobacter* treatment. Be that as it may, for quality spike creation Phosphate solubilising microorganisms was discovered more compelling. It was found that the treatment of the corms with the biofertilizers expanded the aggregate rhizospheric bacterial populace. The action of rhizospheric microbes upgraded the change in different characters of *Gladiolus*.

(Srivastava and Govil, 2005). *Azotobacter* immunization was discovered valuable as far as expanded grain and straw yield and higher nitrogen use effectiveness over the uninoculated medicines of oat assortment JHO-822 and OS-7 (Jatasara et al., 2005).

**PHOTOSYNTHETIC PIGMENT CONTENT**

The shades all out chlorophyll and carotenoids content demonstrated a declining pattern with expanding centralizations of paper factory emanating. Be that as it may, there was a collection of anthocyanin.

The photosynthetic shades, for example, chlorophyll a, b, all out chlorophyll and carotenoid substance were expanded in the plants with expanding convergences of *Azotobacter* (Table 2). The most extreme increment in absolute chlorophyll substance was around 48%, 49%, 58% and 64% than when they were treated with paper factory gushing alone.

A lessening in anthocyanin substance was seen in the plants when they were dealt with with *Azotobacter*. Expanded populace of N<sub>2</sub> settling microscopic organisms in the rhizosphere prompted expansion of chlorophyll colors took after by increment of aggregate dissolvable sugar.

**Table 1: Retrieval Effect of Paper Mill Effluent with *Azotobacter* on the Photosynthetic Pigment Content of *Eleusinecoracana*, Gaertn.**

S. No.	Parameters	Control (water)	Effluent 30% (v/v)	1.5% (w/v)	2% (w/v)	2.5% (w/v)	3% (w/v)
1.	Chlorophyll a (mg/gLFW)	2.42 (100)	1.64 (68)	2.83 (117)	2.88 (119)	3.129 (129)	3.250 (134)
2.	Chlorophyll b (mg/gLFW)	1.94 (100)	1.3 (67)	2.21 (114)	2.17 (112)	2.313 (119)	2.468 (127)
3.	Total Chlorophyll (mg/gLFW)	4.370 (100)	2.949 (67)	5.05 (115)	5.06 (116)	5.442 (125)	5.718 (131)
4.	Carotenoid (mg/gLFW)	2.149 (100)	1.992 (80)	2.32 (108)	2.44 (114)	2.514 (117)	2.707 (126)
5.	Anthocyanin (mg/gLFW)	1.023 (100)	1.411 (138)	0.85 (84)	0.83 (82)	0.787 (77)	0.757 (74)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control.

**BIOCHEMICAL CHARACTERISTICS**

A profound lessening was seen in all the biochemical qualities considered with paper factory gushing aside from infree amino corrosive, L - proline, leaf nitrate and phenol content in *E.coracana*, but the Bio remediated paper plant profluent indicated critical expansion altogether dissolvable sugar and leaf protein substance (Table 2).

It was accounted for that vermicompost fortifies plant supplement uptake and digestion system and the positive impact on sugar substance was 24% over control (Jeyaraj et al., 1994). *Azotobacter* within the sight of 75 kg N ha<sup>-1</sup> were found to have altogether enhanced the development, yield parameters and nitrogen content in *Allium cepa* knob (Tesfaye Balemet et al., 2007).

The protein substance was likewise expanded after treatment with biofertilizer incorporated paper plant profluent. It was at the rate of 94%, 98%, 123% and 132% as for control for (1.5%, 2%, 2.5% and 3% (w/v)) *Azotobacter*, blended with 30% paper factory emanating. Purakayastha and Bhayanagal (1997) reported the expansion of protein and decline of proline in vermicompost revision. Aggregation of dissolvable leaf protein meant that high metabolic status of Nitrogen. The free Amino corrosive and nitrates was lesser with biofertilizer treatment. The Bio remediated paper plant emanating enhanced the treated *E.coracana* plants by recuperating from the anxiety and thus the lessening of proline to the most extreme. Stewart and Lee (1974) reported that the aggregation of proline was a versatile reaction to push. Diminishing substance of anthocyanin along these lines diminished the measure of phenol in bio treated. Anthocyanin goes about as a controller for the union of phenol (Zamprometov and Zagoskina, 1987). *Azotobacter* integrates auxins, cytokinins, and GA-like substances, and these development materials are the essential substances controlling the improved development (Sartajet al., 2004).

Amino corrosive, proline, leaf nitrate and aggregate phenol substance were diminished with expanding convergences of *Azotobacter* coordinated with paper factory emanating. The diminishment in their amino corrosive, proline, leaf nitrate and aggregate phenol was high in 3%(w/v) *Azotobacter*. *Azotobacter* is tolerant to high salts. Bio compost enhances soil ripeness and upgrades supplement uptake and water uptake, in this manner supporting in better foundation of plants (Jatasaraet al., 2005).

**Table: 2. Effect of Paper Mill Effluent with *Azotobacter* on the Biochemical Characteristics of *Eleusine coracana*, Gaertn**

S. No.	Parameters	Control water	Effluent 30% (v/v)	1.5% (w/v)	2% (w/v)	2.5% (w/v)	3% (w/v)
1.	Total soluble sugar (mg/g LFW)	13.41 (100)	7.51 (56)	12.87 (96)	13.81 (103)	15.28 (114)	16.89 (126)
2.	Protein (mg/g LFW)	13.53 (100)	9.06 (67)	12.71 (94)	13.25 (98)	16.64 (123)	17.85 (132)
3.	Amino acid (μ mole/g LFW)	3.245 (100)	4.89 (151)	3.75 (115)	3.45 (107)	3.11 (96)	2.60 (80)
4.	Nitrate (mg/g LFW)	256.5 (100)	325.5 (127)	251.5 (98)	236 (92)	223 (87)	210 (82)
5.	Proline (mg/g LFW)	1.153 (100)	1.42 (123)	1.222 (123)	1.118 (197)	0.956 (83)	0.876 (76)
6.	Phenol (mg/g LFW)	3.26 (100)	4.423 (136)	3.39 (104)	2.99 (92)	2.83 (87)	2.41 (74)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control.

**ENZYME ACTIVITIES**

The differences in the biochemical content are directly related to the differences in the metabolism, which in turn, depends on the enzyme activity. Hence, some major enzyme activities were analysed and the enzyme nitrate reductase, catalase and peroxidase activities were significantly reduced.

The in vivo nitrate reductase activity was increased with the increase in the concentration of bio

remediated paper mill effluent (Table 4). It was at the rate of 17%, 29%, 47% and 58% for(1.5%, 2%, 2.5% and 3% (w/v)) *Azotobacter* respectively mixed with 30% paper mill effluent than when the plants were treated with effluent alone.

The activities of catalase and peroxidase were found to be decreased with the increase in concentrations of the bio fertilizer. The enzyme catalase activity was reduced to about 71%, 17%and 81% respectively with 3% (w/v) *Azotobacter* respectively than when the plants were treated with effluent alone. The peroxidase enzyme also followed a negative trend as catalase, when the biofertilizer integrated with paper mill effluent was treated on *E.coracana*. Peroxidase is a metalo enzyme containing porphyrin bound ion. It acts on a wide range of substrates such as phenol, aromatic amines and inorganic compounds, which led to destruction of IAA, degradation of chlorophyll and inhibition of photo oxidation in plants ( BalaSingha, 1982).

**Table 3: Effect of Azotobacter with Paper Mill Effluent on the Enzyme Activities of Eleusinecoracana, Gaertn**

S. No.	Parameters	Control (water)	Effluent 30% (v/v)	1.5% (w/v)	2 % (w/v)	2.5% (w/v)	3% (w/v)
1	Nitrate reductase (μ mole/g LFW)	581.427 (100)	406.9 (70)	505.8 (87)	575.6 (99)	680.2 (117)	744.2 (128)
2	Catalase activity (μ mole/g LFW)	2.834 (100)	4.61 (163)	3.74 (132)	3.54 (125)	2.94 (104)	2.46 (87)
3	Peroxidase activity (μ mole/g LFW)	0.389 (100)	0.552 (142)	0.494 (127)	0.412 (106)	0.361 (93)	0.346 (89)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control.

**CONCLUSION**

The Biofertilizers *Azotobacter* remediated the paper mill effluent and increased the growth and biochemical parameters of *E.coracana* when it was compared with control plants treated with water and effluent alone.

**BIBLIOGRAPHY**

- 1.Addy, S.K. & R.N. Goodman, 1972. Indian Phyto path. 25: 575-579.
- 2.Balasinha, D. 1982. Regulation of peroxidase in higher plants - A review, plant physiol and Biochem., 9(2): 140-143.
- 3.Bates, L. S., Waldren, R. P. & I. D. Teare, 1973. Determination of the proline in water stress studies. Plant and Soil.39: 205-208.
- 4.Bray, H.G. & W.V. Thorpe. 1954. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal.1: 27-52.
- 5.Chaykovskaya, L.A., Patyka, V.P. & T.M. Melnychuk 2001. Phosphorus mobilizing microorganisms and their influence on the productivity of plants. In (W.J. Horst. Eds.). Plant Nutrition- Food Security and Sustainability of Agro ecosystems. 668-669.
- 6.Chetti, M.B., Antony, E., Mummigatti, U.V. & B. Dodamani, 1995. Role of nitrogen and Rhizobium on nitrogen utilization efficiency and productivity potential in groundnut genotypes. Farming Systems. 11(12);

209-216.

7. Goldstein A.H. 1986. Bacterial phosphate solubilization: Historical perspective and future prospects. *Am.J. Alt. Agric* 1, 57-65.

8. Gyaneshwar P., Naresh Kumar G. and Parekh L.J. 1998a. Effect of buffering on the phosphate solubilizing ability of microorganisms. *World J. Microbiol. Biotechnology*. 14. 669-673.

9. Jayaraman, J., 1981. Laboratory manual in biochemistry, Willey Eastern Company Limited, Madras, 1-65.

10. Jatasara, D. S. Rana, R. S. Sheoran. Efficacy of *Azotobacter* inoculation under graded doses of nitrogen fertilizer in relation to growth, yield and nitrogen utilization efficiency of oat (*Avena sativa* L.) *Journal Acta Agronomica Hungarica*, 48(2), September 2000 DOI 10.1556/AAgr.48.2000.2.6. Pages 165-170. Online Date Friday, July 22, 2005.

11. Jaworski, E.G, 1971. Nitrate reductase assay in intact plant tissues. *Biochem Biophys. Res. Commun.* 43: 1274-1279.

12. Jeyaraj, K., Chellapandi, S., Ramasubramanian, V. & G. Vijayalakshmi, 1994. Impact of cardboard and plate making effluents and its retrieval by vermicompost on the growth of *Eleusine coracana*, G. *Proc. Natl. Conf. Modern trends in Plant Sci.*, AVCCollege, Mannampandal, Mayiladuthurai, pp. 106-109.

13. Kar, M. & D. Mishra, 1976. Catalase, Peroxidase and Polyphenol- Oxidase activities during rice leaf senescence, *Plant Physiol* 57: 315-319.

14. Lowry, Od.H., Roseburg, N.J., Farr, A.L. & R.J. Randall, 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.* 193: 262-275.

15. Mohammad Iris Accepted: 10 February 2006. Published online: 5 April 2006. Effect of Integrated Use of Mineral, Organic N and *Azotobacter* on the Yield, Yield Components and N-nutrition of Wheat (*Triticum aestivum*, L.) Source: *Pakistan Journal of Biological Sciences* 6 (6): 539-543, 2003.

16. Moraes, S.G., N. Durán & R. S. Freire. Remediation of Kraft E1 and black liquor effluents by biological and chemical processes *Journal Environmental Chemistry Letters*, 4(2), June, 2006. Pages 87-91 Springer Link Date Wednesday, April 05, 2006.

17. Morre, S. & W. H. Stein, 1948. Photometric method for use in the chromatography of amino acids. *J. Biol. Chem.* 176: 367-388.

18. Kalugasalam, A. 1981. Studies on phosphorus microorganism and certain physical, chemical and biological factors influencing phosphorus solubilization. M.Sc. (Agri) Thesis. TNAU, Coimbatore. p. 99.

19. Mariappan, V. 2002. Evaluation of treated tannery effluent and soil amendment studies for growing certain tree species. Ph.D. thesis, Gandhigram Rural Institute, Deemed University, Dindigul, Tamil Nadu.

20. Naidu, P.H., 2000. Response of bunch varieties of groundnut to *Rhizobium* inoculation. *Leg. Res.* 23(2); 130-132.

21. Purakayastha, T.J. and R.K. Bhayanagar, 1997. *Indian Farming*, 46(11): 35- 37.

22. Sekar, R., Thangaraju, N. and R. Rangasamy, 1995. *Phykos*. 34:49-53.

23. Srivastava, R. M. Govil. *SHS Acta Horticulturae* 742: International Conference and Exhibition on Soilless Culture: ICESC 2005 Influence of biofertilizers on growth and flowering in gladiolus cv. american beauty.

24. Stewart, G.R. and J.A. Lee, 1974. *Planta*, 120: 279-289.

25. Subbarao, G., Johanson, V., Kumar Rao, C. and M.K. Jana, 1990. In: *Biofertilizers in Agriculture*. Oxford IBH Publishing Co., New Delhi. pp. 85-90.

26. Sudhakar, P., Chattopadhyay., G.N., Gangwar., S.K. and J.K. Ghosh, 2000. Effect of foliar application of *Azotobacter*, *Azospirillum* and *Beijerinckia* on leaf yield and quality of mulberry (*morus alba*). *J. Agri. Sci.* 134: 227-234.

27. Tesfaye Balemi, Netra Pal, Anil Kumar Saxena. 2007. Response of onion (*Allium cepa* L.) to combined application of biological and chemical nitrogenous fertilizers. *J. Acta agriculturae Slovenica* Publisher Versita p-ISSN 1581-9175, e-ISSN 1854-1941, Vol. 89, No. 1, August 2007 Category research article DOI



10.2478/v10014-007-0013-y. Page 107-114 Online Date Friday, December 07, 2007.

28.Wellburn, A.R. & H. Lichtenthaler, 1984. In: Advances in photosynthesis Research (ed. Sybesma) Martinus Nijhoff, Co., The Hague. pp: 9-12.

29.Zahir Ahmad Zahir, Syed Anjum Abbas, Muhammad Khalid, Muhammad Arshad, 2000. Substrate Dependent Microbially Derived plant Hormones for Improving Growth of Seedlings. PakistanJ. Bio Sci. 3 (2) 289-291.

30.Zamprometov, M.N. & N.V. Zagoskina, 1987. Fiziol. Rast. 34: 165-168.