Azotobacter Chroococcum Mass Culture for Production of Bio-Fertilizer, Its Sustained Efficacy on Nitrogen Fixation and Crop Productivity in Mulberry Garden

[1,]S.Rajaram , [2,]Klisdamon Nongrang , [3,]S.K.Mandal, [4,]M.K.Ghosh and B.B.Bindroo.

[1,2,3,4]Central Sericultural Research and Training Institute, Central Silk Board, Berhampore - 742 101, Murshidabad, West Bengal, India.

Abstract :

Mulberry is cultivated by farmers for its leaves, the sole food for silkworm (*Bombyx mori* L.) for commercial production of raw silk in Sericulture Industry. As mulberry is a perennial crop can be maintained for several years in the field, selection of suitable land and follow-up of recommended package of practices are inevitable for maintenance of potential productivity of the variety selected for cultivation. As the quality of mulberry leaves alone contributes about 38.2% for the success of silkworm cocoon crop, quality linked leaf productivity of mulberry leaves can be achieved through adequate supply of all required input into soil. Nitrogen is one of the important macronutrient required for mulberry in larger quantity. In order to reduce the high cost involved towards nitrogenous chemical fertilizers and to maintain the soil health in an eco-friendly way Integrated Nutrient Management (INM) approach in agriculture sector became popular and the same has been followed in mulberry cultivation as well in recent years. Use of different kinds of microbial inoculants as bio-fertilizer to fix atmospheric nitrogen in mulberry garden brought improvements in soil health maintenance and helps to reduce nitrogenous chemical fertilizer requirements and expenditure to farmers considerably without affecting the quality linked productivity.

Keeping in view of the above an experiment study was conducted to ascertain the consistent efficacy of *Azotobacter chroococcum* inoculants @ 10^{8-9} cells per g charcoal carrier material used as nitrofert bio-fertilizer application in mulberry garden during July to September 2012 crop. S1635 mulberry variety in Paired Row System [PRS] of plantation with (150+90) x 60 cm spacing under irrigated condition with two treatments i.e., T1 as control with basal dose of application of 20 MT FYM ha^{-1} $year^{-1}$ in two split doses and recommended 336:180:112 NPK ha^{-1} $year^{-1}$ in 5 split doses and in T2, except 50% of N replaced by 20 kg nitrofert bio-fertilizer ha^{-1} $year^{-1}$ in 5 split doses all other nutrients and package of practices as followed in T1 with 13 replications in CRD. Average leaf yield of 7.35 & 7.34 and total biomass of 12.95 & 13.0 tons ha^{-1} obtained in T1 and T2 respectively and quality of leaves on economic characters found without significant difference between the treatments revealed the consistent efficiency of *Azotobacter chroococcum* in fixing atmospheric nitrogen in the soil of mulberry garden to reduce nitrogenous chemical fertilizer and expenditure without affecting the quality linked leaf productivity and mass culture of the bacteria for preparation of Nitrofert bio-fertilizer, its application techniques are discussed in the paper.

Key Words : Mulberry leaf, bio-fertilizer, biological nitrogen fixation, eco-friendly soil health, potential productivity.

1. Introduction :

Mulberry is cultivated by farmers for its leaves, the sole food for silkworm (*Bombyx mori* L) for commercial production of raw silk in Sericulture Industry. As mulberry is a perennial crop can be maintained for many years, selection of land and follow-up of recommended package of practices are inevitable for quality linked potential productivity throughout. Further the quality of mulberry leaves as single factor contributes about 38.2% for the success of silkworm crop (Miyashita, 1986), adequate supply of all required input into soil is very much essential. In India, during the Green Revolution period more emphasis was given for increase unit area productivity of crops which facilitated in indiscriminate application of inorganic chemical fertilizers, chemicals to control various pests and diseases without considering the soil health maintenance for long-term use for agriculture purposes resulted in considerable damage to the soils of agriculture land. It was reported that out of 235 mha of cultivable area, soils of 166 mha have been damaged (Swaminathan, 1994) in the country, necessitated alternate methods to improve the soil health. Excessive uses of nitrate and phosphatic fertilizers have led to extensive contamination of surface and ground waters (Dahama, 2003). To complete life cycle normally, living organism requires a large numbers of substances from outside are called nutrition. Green plants being autotrophic, requires only inorganic substances from outside (Pandey and Sinha, 1972). An essential element is defined as one whose absence prevents plants from completing its life cycle or one that has clear

physiological role (Arnon and Stout, 1939). Though the atmosphere contains more than 70% of nitrogen, only about 0.1% of fixed nitrogen is present in the soil and small traces of nitrogen from the atmosphere reach the soil in a dissolved state in rain water (Rangaswami and Bagyaraj, 2004). The fate of nitrogen fertilizers in the soil is controlled by several physical, chemical and biological factors. The percentage of recovery of nutrients varies between the different types of fertilizers reported as 50-60; 5-15 and 75% of N P K respectively and nitrogen deficiency is observed in plants grown on soils with low organic matter (< 0.4 % organic carbon) and also reported that nearly 62 % soils are deficient in Nitrogen (Anonymous, 2011).

Nitrogen fixation is the reduction of N2 (atmospheric nitrogen) to NH3 (ammonia). Free living prokaryotes with the ability to fix atmospheric dinitrogen (diazotrophs) are ubiquitous in soil. But our knowledge of their ecological importance and their diversity remains incomplete. In natural ecosystems, biological N2 fixation is most important source of N. The capacity for nitrogen fixation is widespread among bacteria and archaea. The estimated contribution of free-living N-fixing prokaryotes to the N input of soil ranges from 0-60 kg. ha^{-1} $year^{-1}$ (Bűrgmann *et al.,* 2003). Bio-fertilizers can make significant contribution towards the development of strategies for productivity improvement which do not lead to an exponential rise in the consumption of non-renewable forms of energy (Subba Rao, 1982) and the use of bio-fertilizers is currently gaining interest as a cheap, safe alternative to chemical fertilizers (Sharma, 2002).

After isolation of two aerobic free living nitrogen fixing bacteria in 1901 by Martinus Beijerinck, and named them as *Azotobacter chroococcum* and *A. agile* study on practical applicability of these *Azotobacter* spp. attracted several workers. Yamagata and Itano (1923) reported that *Azoto-bacter* is ubiquitous in neutral and weakly basic soils, but not in acidic soils and the growth of bacteria favored at a temperature of 20-30°C and Moreno *et al.,* (1986) observed that in dry soils, *Azotobacter* can survive upto 24 years in the form of cysts. Use of variety of carbohydrates, alcohols and salts of organic acids as sources of carbon and pH 4.8 to 8.5 found optimum for growth of the bacteria George (2005).

Wong and Maier (1985) reported that hydrogen dependent mixotrophic growth of *Azotobacter* in a nitrogen-free medium containing mannose and availability of hydrogen in the soil facilitates the growth of *Azotobacter* in nature. Culture media inoculated with *A. chrococcum* with high NaCl concentration, incubated on rotary shaker for 60 hours at 200 rpm facilitated full growth of colony and shown brown colour pigment. *A. chrococcum* being *a* non-symbiotic bacteria have a great potential for use in production of bio-fertilizer due to its ability to fix N2 (Nakade *et al.,* (2012). Besides, nitrogen fixation, it also produces, Thiamin, Riboflavin, IAA and gibberellins, when applied to seeds, seed germination is improved to a considerable extent and also controls plant diseases due to the above substances (Kader *et al.,* 2002).

Presence of ferredoxin, hydrogenase and an important enzyme nitrogenase required for nitrogen fixation was reported by Shank *et al.,* (2005) and of the different type of nitrogenase, the basic one is Molybdenum- iron nitrogenase was reported by Howard and Rees (2006). Chen *et al.,* (1995) observed that *Azotobacter* spp. facilitate the mobility of heavy metals in the soil and thus enhance bioremediation of soil from heavy metals, such as cadmium, mercury and lead. Emtiazia *et al.,* (2004) reported that *Azotobacter* biodegrade chlorine-containing aromatic compounds, such as 2,4,6-trichlorophenol which was used as an insecticide, fungicide and herbicide till its mutagenic and carcinogenic effect was found (Li *et al.,* 1991).

Like other agricultural crops, mulberry requires all sixteen nutrients and nitrogen in large quantity. Based on the high cost involved in application of nitrogenous chemical fertilizers and to maintain the soil health in an eco-friendly manner, Integrated Nutrient Management (INM) approach in agriculture sector became popular in recent years, the same has been followed in mulberry cultivation as well.

A. chroococcum cells blended with peat soil/charcoal/FYM in powdered form as carrier material containing 10^{8-9} cells g^{-1} observed as optimum for application in mulberry garden. CSR&TI., Berhampore, India has standardized the mass culture technique for production of a bio-fertilizer under the name "Nitrofert" to reduce nitrogenous fertilizer requirement and expenditure in mulberry cultivation without affecting the quality linked leaf productivity and for eco-friendly way of soil health improvement (Sudhakar et al., 2000). Mass culture of A. chroococcum for production of nitrofert bio-fertilizer, findings of the experiment conducted on its application and crop productivity in S1635 mulberry garden are discussed in detail in this paper.

2. Materials And Methods

2.1Mass multiplication of *Azotobacter chroococcum* for Nitrofert bio-fertilizer production in laboratory :

Preparation of Walkman's bacterial culture medium and inoculation of *A. chroococcum* mother culture for mass culturing and production of bio-fertilizer in charcoal powder as carrier material maintaining 10^{8-9} cells g^{-1} as described by Sudhakar *et al.*, 2000 rotary shaker for shaking at 200 rpm for 60 hours for mass (**Plates : 1-10**).

2.2 Efficacy of Nitrofert on nitrogenous fertilizer saving and crop productivity in mulberry garden :

The experiment was carried out in **Plot No. A-9** Agronomy Section of CSR&TI, Berhampore West Bengal, India, well established irrigated mulberry garden raised in alluvial soil with S1635 high yielding variety under Paired Row System with plant spacing of (150+90) x 60 cms the mulberry garden during July to September 2012. After pruning of plants, 26 number of plots demarcated each with two paired rows and 10 plants and thus a total 40 number of plants per plot (Chaturvedi and Sarkar, 2000) in Completely Randomized Design [CRD] as described by Sukhatme and Amble (1985) was drawn as experiment plan to accommodate 2 treatments with 13 replications each. In T1, recommended quantity of FYM @ 20 ton ha^{-1} $year^{-1}$ in two equal split doses and chemical fertilizers NPK @ 336 : 180 : 112 kg. ha^{-1} $year^{-1}$ in 5 equal split doses (Ray *et al.*, 1973) as control and in T2 FYM @ 20 ton ha^{-1} $year^{-1}$ in two equal split doses and chemical fertilizers NPK @ 168 : 180 : 112 kg. and Nitrofert 20 kgs ha^{-1} $year^{-1}$ in 5 equal split doses (**Plates : 11-16**). Irrigation water applied as and when required and all other package of practices recommended for mulberry garden maintenance were followed uniformly in all plots. On 70^{th} day 5 randomly selected plants from each plot were pruned and observations made on the following parameters individually and yield was estimated as suggested by Sreenivasa Shetty *et al.*, (1990).

2.3 Growth Parameters

like Number of branches / plant, Branch height (cm), Total shoot length / plant (m), Number of leaves / branch, Number of leaves / plant, Total leaf weight / plant (kg), Total shoot weight /plant (kg), Green biomass weight / plant (kg), Leaf weight ha^{-1} $crop^{-1}$ (ton), Shoot weight ha^{-1} $crop^{-1}$ (ton), Biomass green weight ha^{-1} $crop^{-1}$ (ton), Biomass dry weight ha^{-1} $crop^{-1}$ (ton) and **Leaf quality Parameters** like Moisture Content of leaf (MC) and Moisture Retention Capacity (MRC) (Vijayan *et al.*, 1997). All data of the experiment were subjected to statistical analysis using AGRES Software and the results were tabulated and discussed separately.

3. Results And Discussion

3.1 Mass multiplication of *Azotobacter chroococcum* for Nitrofert bio-fertilizer production in laboratory :

From 10 litres of *Azotobacter chroococcum* bacteria media charcoal powder mixed 23 kgs of Nitrofert bio-fertilizer with 10^{8-9} cells g^{-1} carrier material prepared.

3.2 Efficacy of Nitrofert on nitrogenous fertilizer saving and crop productivity in mulberry garden :

Microbial inoculants in carrier based preparations containing beneficial microorganisms in a viable state intended for seed or soil application and designed to improve soil fertility and help plant growth by increasing the population and biological activity of desired microorganism in the root environment (Subba Rao, 1982). Inoculation of Azotobacter and AM fungus in mulberry has proved beneficial in terms of economizing N and P fertilizer application by 50% without adverse effect on leaf yield and quality (Das *et al*, 1994). Application of n-triacontanol (Vipul) as foliar spray and use of *Azotobacter* bio-fertilizer could increase the leaf yield by 15-20% besides, 50% reduction in nitrogenous fertilizer (Rajanna *et al*, 2005).*Azotobacter* bio-fertilizer application @ 20 kg ha^{-1} $year^{-1}$ was able to curtail 50% nitrogenous fertilizer requirement of mulberry without affecting yield and quality of leaves (Sudhakar *et al*, 2000). Similar results (Table 1 & Fig. 1-2) obtained in the experiment conducted are discussed as below:

3.3 Number of branches / plant : An average of 15.92 and 13.92 number of branches recorded per plant in T2, T1 respectively and the difference between the two treatments was statistically significant @ CD 5% level.

3.4 Branch height (cm) and total shoot length $plant^{-1}$ (m) The difference in the average height of branches in T1 and T2 recorded was statistically non significant @ CD 5% level the same was reflected in as well**.**

3.5 Number of leaves $branch^{-1}$ and $plant^{-1}$: Though the difference in average number of leaves per branch observed was statistically significant @ CD 5% level and the same per plant was non significant in T1 and T2.

3.6 Total leaf weight and shoot weight plant^{-1} (kg) : An average of 0.5285 kg of leaves produced per plant during the crop period in T1 and T2. Similarly, there was no difference in weight of shoots among the treatments.

3.7 Green biomass weight plant^{-1} (kg) : As there was no difference in the weight of leaves and shoots produced per plant during the crop period in T1 and T2 the same was reflected in the total biomass green weight as well.

3.8 Leaf weight ha^{-1} crop^{-1} (ton) : Average estimated yield of 7.35 and 7.34 tons of mulberry leaves ha^{-1} crop^{-1} in T1 and T2 respectively are on par statistically @ CD 5% level.

3.9 Shoot weight ha^{-1} crop^{-1} (ton) : Average estimated shoot weight of 5.55 and 5.67 tons harvested during the crop in T1 and T2 respectively are on par statistically @ CD 5% level.

3.10 Biomass green weight ha^{-1} crop^{-1} (ton) : Average estimated biomass green weight of 12.90 and 13.00 tons ha^{-1} produced during the crop in T1 and T2 respectively and its dry weight recorded are on par statistically @ CD 5% level.

b) Leaf quality Parameters :

3.11 Moisture Content of leaf (%) : Average Moisture Content of leaf of 81.79 and 81.71% recorded during this crop in T1 and T2 respectively are on par statistically @ CD 5% level

3.12 Moisture Retention Capacity of leaf (%) : Average Moisture Retention Capacity of leaf of 94.00 and 94.94% recorded during this crop in T1 and T2 respectively are on par statistically @ CD 5% level.

4. Conclusion :

It may be concluded that the maintenance of *A. chroococcum* bacteria culture and mass multiplication in Walkman's culture medium under laboratory conditions for production of **"Nitrofert" bio-fertilizer** is viable. **"Nitrofert"** bio-fertilizer application @ 20 kgs. ha^{-1} year^{-1} in 5 split doses in mulberry garden helps to reduce upto 50% nitrogenous chemical fertilizer requirement and saves expenditure without affecting the quality linked productivity, in addition it improves the soil health in an eco-friendly way.

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Fig.1 Showing Leaf, shoot & biomass weight

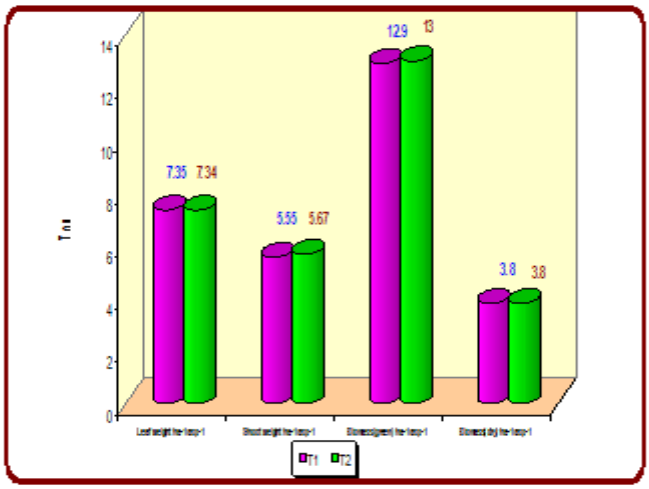

Fig. 2 Showing Moisture Content & Moisture Retention Capacity of leaf

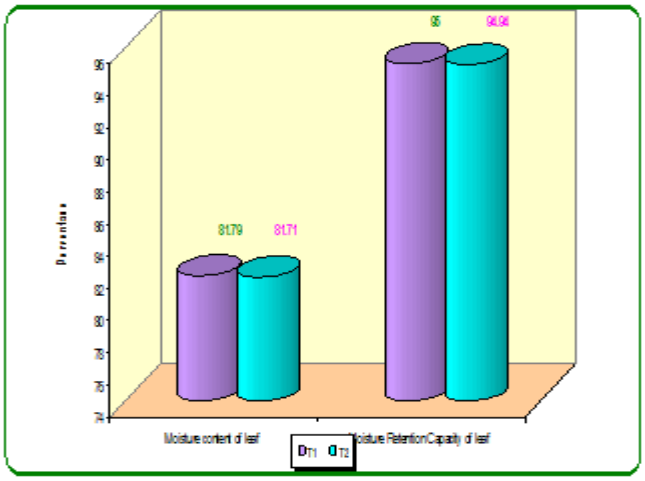

Table : Showing the effect of *A.chroococcum* bio-fertilizer (Nitrevit) on quality and productivity of mulberry Vs. recommended dose of chemical fertilizer

Treatment	Growth parameters													Quality parameters		
	No. of branches / plant	Height of branch (cm)	Total shoot length / plant (m)	No. of leaves / branch	No. of leaves / plant	Leaf weight / plant (kg)	Shoot weight / plant (kg)	Total green biomass weight / plant (kg)	Leaf weight ha^{-1} crop^{-1} (ton)	Shoot weight ha^{-1} crop^{-1} (ton)	Total green biomass weight ha^{-1} crop^{-1} (ton)	Total dry biomass weight ha^{-1} crop^{-1} (ton)	Moisture Content of leaf (%)	Moisture Retention Capacity of leaf (%)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
T1	13.92	120.62	16.70	20.75	283.57	0.53	0.40	0.93	7.35	5.55	12.90	3.80	81.79	85.00
T2	15.92	113.54	17.94	18.63	291.45	0.53	0.41	0.94	7.34	5.67	13.00	3.82	81.71	84.94
Grand Mean	14.9235	117.0796	17.321 5	19.6892	287.5079	0.5285	0.405	0.9331	7.3412	5.6469	12.9804	3.8092	81.7527	84.9742
SEd	0.919	3.7835	1.0557	0.8383	12.931 9	0.0395	0.0397	0.0771	0.5486	0.5394	1.069	0.3145	0.2125	0.1323
CD @ 5% level	1.8959	7.8031 NS	2.1781 NS	1.7302	26.682 NS	0.0815 NS	0.0820 NS	0.1590 NS	1.1323 NS	1.1132 NS	2.2063 NS	0.649 NS	0.4384 NS	0.2731 NS

Std : Standard Error deviation; CD : Critical Difference; * Significant at CD 5% level; NS: Non significant

Plates showing different stages in mass multiplication of Azotobacter chroococcum and Nitrofert biofertilizer production

Plates : 1 & 2 Culture media preparation

Plate: 3 Sterilization of culture media

Plate : 4 Inoculation of *A. chroococcum*

Plate : 5 *A. chroococcum* culture

Plate : 6 Shaking for growth of culture

Plate : 7 Microscopic observation

Plate : 8 Mixing of culture in carrier material

Plates showing Nitrofert biofertilizer production & experiment in the field

Plate : 9 Nitrofert weighing & packing

Plate : 10 Nitrofert Bio-fertilizer

Plate : 11 Experiment Plot layout

Plate : 12 Mixing of NPK fertilizer

Plate : 13 Nitrofert mixing with FYM

Plate : 14 Treatment in progress

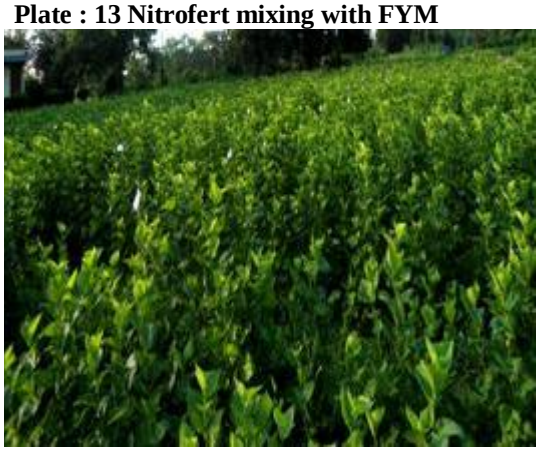

Plate : 15 Experimental plot

Plate : 16 Data collection