Research Article

Comparative Study of Liquid Biofertilizer and Carrier Based Biofertilizer on Green Leafy Vegetables

N.Uma Maheswari*, M. Kalaiyarasi
PG and Research Department of Microbiology, SengamalaThayaar Educational Trust, Womens College, Mannargudi, Tamil Nadu, India.
***Corresponding author's E-mail:** umasamyamf@gmail.com

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ABSTRACT

The high cost of fertilizer production and environmental pollution caused by the use of these fertilizers makes necessary to use other sources especially biofertilizers. The biomass of the bacterium *Azotobacter* can be used as a biofertilizers due to its ability to fix nitrogen from the atmosphere. The purpose of this research was to study the survival of bacteria *Azotobacter* on liquid and carrier using pot culture. T_1 – Liquid form of *Azotobacter spp.* T_2- Carrier based *Azotobacter*, T_3- Combined form of liquid and carrier based *Azotobacter,* C-Control. Hence our study was clearly highlighted that combined inoculation liquid biofertilizer and carrier based biofertilizer such as Azotobacter could enhance the morphological parameters at 20th day such as height of the plant (22±2.84cm) number of leaves (19±1.2), shoot length (24±0.89cm), root length (8.46±0.56cm).number of roots and biochemical constituents such as chlorophyll, carotenoids, compared to individual inoculation and control.

Keywords: Biofertilizer, inoculation, liquid, carrier

INTRODUCTION

The biofertilizers have the ability to convert nutrition important elements from unavailable to available from through biological processes[1] some bacteria are capable to fix the atmospheric nitrogen. The free-living non-symbiotic nitrogen-fixing bacteria those belonging to genus *Azotobacter* sp. which is a heterotrophic, arobic microorganism being broadly dispersed in different environments, such as soil, water and sediments.[2]

Different investigation stated that inoculation with *Azotobacter sp*. has beneficial effects on plant yield[3] due to the increase in fixed nitrogen content in soil and to the microbial secretion of stimulation hormons, gibberellins, auxins and cytokinins and established the ability of *Azotobacter* sp. to solubilizing phosphates.

Carriers should have near neutral are readily adjustable P^H, be abundant locally at a reasonable cost and able to sterilize. These properties only indicate the potential for a good carrier, while final selection for carrier must be based on microbial multiplication and survival during storage, and general method of planting, equipment used for planting, acceptable cost[4].

Characteristics of *Azotobacter*[5]

Azotobacter is a free–living, gram negative, aerobic, nitrogen-fixing bacterium and is therefore being used as biofertilizer to replace chemical fertilizers. It grows from 28°C-30°C and a pH range 7.0 to 7.5.

It uses sugar, alcohols and salts of organic acid for growth. Generally it fixes non–symbiotically about 10mg of atmospheric nitrogen/gm of carbohydrates (usually glucose) consumed. It is non-spore forming but can form cyst in adverse conditions and in older cultures grown with sugar as the carbon source.

Carrier Material[6]

Various types of material are used as carrier for seed or soil inoculation. For preparation of seed inoculant, the carrier material is fine powder with particle size of 10-40µm. According to the "Handbook for Rhizobia", the properties of a good carrier material for seed inoculation are: i)Non–toxic to inoculant bacteria strain ii) Good moisture absorption capacity iii) Easy to process and free of lump-forming material iv) Easy to sterilize by autoclaving or gamma-irradiation V) Available in adequate amount. vi) Inexpensive Vii) Good adhesion to seed. Viii) Good pH buffering capacity. Needless to say. ix) Non–toxic to plant, is another important property.

Liquid form of biofertilizer[7]

Liquid biofertilizer is increasingly available in the market as one of the alternative to chemical fertilizer and pesticide. One of the benefits from biofertilizer is a contribution from population of microorganism available.

Traditionally liquid biofertilizer produced from fermentation of effective microorganism (EM) was recommended to be used with in three months. Nowdays the production of ready to use liquid biofertilizer from EM is becoming available in market.

The aim of the work is to investigate the comparative study of liquid biofertilizer and carrier based biofertilizer on green leafy vegetables.

MATERIALS AND METHODS

Soil Sample Collection

The Soil samples were collected from paddy field at vilakkudi in Thiruvarur district using sterile polythene bag.

Isolation Of *Azotobacter* From Soil Sample[8]

Azotobacter was isolated from the soil sample by serial dilution as 1.0g of air dried samples was dissolved in 99ml of distilled water. The soil suspension was further diluted up to 10^6 level. The diluted soil suspension (0.1ml) was spread on the surface of Jensen agar medium which is a selective medium for isolating *Azotobacter.* The p^H of the medium was adjusted to 7.0 with the help of 1N HCl/1N NaOH. The plates were incubated at 28°C for 5-7 days and the colonies were observed. Strains of *Azotobacter* were picked out and purified by repeated streaking on Jensen medium and were preserved as slant culture for further usage. Large mucoid opaque colonies were observed after 5-7 days.

Biochemical Constituents

Chlorophyll, total chlorophyll, carotenoids were also estimated[9,10].

Pot Culture Technique

A pot culture experiment was conducted in garden soil with the following treatment. Plants were used for this investigation namely green leaf vegetables. The plants were treated with *Azotobacter*, and respectively by the following method.

Seed Treatment

The seeds were soaked in *Azotobacte*r, culture for over night and pot culture were carried out

Treatment

There were 4 treatments resulting from combination of

T_1 – Liquid form of *Azotobacter spp*.

T_2 - Carrier based *Azotobacter.*

T_3- Combined form of liquid and carrier based *Azotobacter.*

C-Control

There were four replications for each treatment. The pots were maintained in the open shade at the temperature of 27°C-30°C.

Morphological Parameter

After 7th, 15th, and 20th days of growth, 3 plant for per pot were removed from all sample, and studied for the following morphological parameters they were, Height of the plant (in cm), Number of leaves (per plant), Number of roots (per plant), Root length (in cm), Shoot length (in cm).

Analysis of Nutrient Status

Nitrogen ,phosphorus, potassium, were also estimated by standard method.[11-13]

RESULTS

The effect of biofertilizer treatment on vegetative growth of green leafy vegetables was significantly higher in combined inoculation of liquid and carrier based *Azotobacter* (T3) than control. A significant variation in plant height and number of leaves due to application of biofertilizer was noticed. Statistically significant increase in plant height, number of leaves, shoot length, root length, number of roots.

Isolation and Identification of Bacteria

Soil sample was collected from Vilakkudi, ThiruvarurDistrict, Tamil Nadu, South India. *Azotobacter sp* were isolated from the soil sample by serial dilution technique by streak plate method.

In the gram staining technique gram negative and motile bacteria were observed. In was confirmed by different biochemical test, the identified bacterial colonies are *Azotobacter*. The identified organisms are able to produce separate colonies on different culture media.

Pot culture Treatment

The soil sample was subjected to Tamilnadu, Government Agriculture Department, mobile test centre, Thiruvarur. The physicochemical parameters such as p^H (6.4), nitrogen (0.06 ppm), pottasium (39 ppm) ,Phosphorus (84.25 ppm) were tested for before and after treatment.

Height of the Plant

Combined inoculation of liquid and carrier based Azotobacter (T3) in 20thday the yield concepts such as height of the plant (23±2.84cm) was noted in (Table-1) followed by other treatments and control (Table-2). In 20th day liquid biofertilizer of *Azotobacter* (T1) was agreed with above said response in height of plants (22.5±1.80cm) followed by other treatments and control (Table-1).

Number of Leaves

Combined inoculation of liquid and carrier based Azotobacter (T3) in 20thday the yield concepts such as height of the plant (20.6±1.2cm) was noted in (Table-4) followed by other treatments and control (Table-2).In 20th day liquid biofertilizer of *Azotobacter* (T1) was agreed with above said response in height of plants (20.4±3.39cm) followed by other treatments and control (Table-1).

Shoot Length

Similar observations were made, combined inoculation of liquid and carrier based *Azotobacter* (T3) in 20th day was showed better response in shoot length of plants (18.5±0.89cm) followed by other treatments and control (Table-1). In 20th day carrier based *Azotobacter* (T2) was

exhibited better response in shoot length plants (15.6±6.74cm) followed by other treatments and control.

Root Length

The comparative account of overall treatments combined inoculation of liquid and carrier based *Azotobacter* (T1) in 20th day was showed better response in root length of plants (8.46±0.56cm) followed by other treatments and control (Table-1). In 20th day liquid biofertilizer alone *Azotobacter*(T1) was exhibited better response in root length plants (7.55±0.96cm) followed by other treatments and control (Table -1).

Estimation of Chlorophyll

In 20th day, among the overall treatments combined inoculation of liquid and carrier based *Azotobacter* (T3) was showed higher activity in Chlorophyll-a (0.643± 0.56 mg/g) ,Chlorophyll-b (0.0598± 0.087 mg/g) and total Chlorophyll content (0.0678±0.087 mg/g) of plants followed by other treatments and control (Table-2). T1, T2, alone in 20th day was exhibited better response in Chlorophyll-a, Chlorophyll–b, and total Chlorophyll content followed by control.

Carotenoids content in Liquid Biofertilizer Treatment

In 20th day, among the overall treatments combined inoculation of liquid and carrier based *Azotobacter* (T3) was showed higher activity in Cartenoids of plants (0.598± o.67 mg/g) of plants followed by other treatments and control (Table-2). T1, T2, alone in 20th day was exhibited better response in followed by control.

Hence our study was clearly highlighted that combined inoculation liquid biofertilizer and carrier based biofertilizer such as *Azotobacter* could enhance the morphological parameters such as height of the plant, number of leaves, shoot length, root length, number of roots and biochemical constituents such as chlorophyll, carotenoids, compared to individual inoculation and control. This could be the collective effect of liquid biofertilizer and also reduce the use of chemical fertilizer. Inoculation with plants promoting rhizobacteria (PGPR) may enhance crop productivity either by making other nutrients available or protecting plants from pathogenic microorganisms.

Table 1: Impact of liquid biofertilizers on morphological parameters in *Amaranthusretroflexus* (20thday)

Treatment	Height of the plant (cm)	Number of leaves (cm)	Shoot length (cm)	Root length (cm)
T1	17 ± 1.80 cm	16 ± 3.39 cm	17.3 ± 1.52cm	7.55 ± 0.96cm
T2	15 ± 2.57 cm	15±3.2 cm	15.6 ± 6.74 cm	7.46 ± 0.89 cm
T3	22 ± 2.84 cm	19 ± 1.2 cm	24 ± 0.89 cm	8.46 ± 0.56 cm
C	15.6 ± 6.74 cm	11.7 ± 2. 02 cm	16 ± 0.71 cm	6.15 ± 0.25 cm

Values are triplicates represented as mean ± standard deviation

Table 2: Biochemical constituents in *Amaranthusretroflexus* (20th day)

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)
T-1	0.453±0.43	0.0389±0.86	0.0452±0.12	0.423±0.56
T-2	0.410±0.028	0.355±0.43	0.480±0.080	0.389±0.86
T-3	0.643±0.56	0.0598±0.067	0.678±0.087	0.484±0.049
C	0.0321±0.09	0.0311±0.076	0.412±0.56	0.311±0.076

Values are triplicates represented as mean ± standard deviation

CONCLUSION

Overall, our study was showed the isolation and identification of Azotobacter that has the potential to be used as a biofertilizer. These attributes of the isolate will be of great advantage in agriculture field next trial play a vital role in plant growth promotion, disease suppression and sub sequent enhancement of yield. Liquid biofertilizer have the capacity to replace the traditional chemical fertilizer and carrier based biofertilizer play a major role in restoring soil health. But a lot of measures in terms of technology, Government support, and constructive awareness by well trained technicians one needed.

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