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Research article

EFFECT OF BIOFERTILIZERS ON THE GROWTH OF *Helianthus annuus*

Dhanasekar, R[1] and R.Dhandapani[1]*

Fermentation Technology Laboratory, Department of Microbiology, Periyar University, Periyar Palkalai Nagar, Salem-11, Tamil Nadu, India.

***Corresponding author:** E – mail: danpani@gmail.com*

ABSTRACT: Sunflower is an important oil crop in India. In the present study the effect of biofertilizers *Azotobacter, Azosprillium, Phosphobacter* and *Rhizobacter* on the growth of *Helianthus annuus* was observed. *Azotobacter, Azosprillium, Phosphobacter, Rhizobacter* were isolated from soil and root nodules, respectively and cultured separately in their corresponding selective media. The efficiency of biofertilizer was checked by treating them with hybrid seeds of *Helianthus annulus* (TCSH-1 and SSH-48). In terms of its growth and yield parameters, it was observed that when compared with control, Microbial biofertilizer showed 90% increase yield and 45.87% in growth in TCSH-1 and 60% increased yield and 40.95% increased growth was observed in SSH-48.

Keywords: *Azotobacter, Azosprillium, Phosphobacter,, Rhizobacter,* Biofertilizer, *Helianthus annuus*

INTRODUCTION

Sunflower (*Helianthus annuus*) is one of the most widely cultivated high quality oil seed crop in world, fourth rank after soybean, palm and rape. It is a temperate zone crop but it can perform well under varying climatic and soil conditions [1]. The oil extracted (48 - 53%) edible from this crop is used for either human consumption or industrial purposes. In India soil fertility is diminishing gradually due to soil erosions, loss of nutrition, accumulation of toxic elements, water logging and unbalanced nutrient compensation. Organic manure and bio fertilizers are the alternate sources to meet the nutrient requirement of crops. Among biofertilizers, benefiting the crops are *Azotobacter, Azosprillium, Phosphobacter* and *Rhizobacter* are very important. Biofertilizer referred to living microorganisms, symbiotic and asymbiotic way of supplying nutrients to plants. The asymbiotic nitrogen fixing bacteria *Azotobacter, Azosprillium, Phosphobacter, Rhizobacter* and lead to significant improvement in crops yield by 15 - 20% while reducing the depletion of soil nutrients [8]. Efficiency of different biofertilizers *Azospirillum*, *Azotobacter* and *Rhizobium* with and without suboptimal levels of N (0, 15 and 75 kg/ha) and recommended level of N revealed that the application of 75 kg N/ha supplemented by *Azospirillum* (or) *Azotobacter* (or) *Rhizobium* was found to be more efficient in influencing the seed yield. It showed a significant increased in SSH-1 and the increased yield was statistically in KBSH-1 as compared to the application of recommended level of 100 Kg N alone/ha [7]. Sunflower oil contains large amount of vitamins (A, D, E and K) and considerable amount of proteins (20 -40%). Biofertilizer can increase the soil fertility, the seed yield and its oil content. The role of biofertilizer in agricultural production is great importance. Inoculation of nitrogen fixing bacteria with biofertilizer is increases the phosphorus level that influence the sunflower seed oil content and the proportion of fatty acids (unsaturated/saturated fatty acids ratio) [1]. Biofertilizers can also plant resistant to adverse environmental stresses. The beneficial effect of *Azotobacter* is to fix the atmospheric nitrogen. It increases the seed germination, plant growth and yield. In certain condition they also exhibit antifungal activities and there by fungal disease may be controlled indirectly. *Azospirillum* also fix the atmospheric nitrogen and stimulates the effect on root development. It has the ability to reduce nitrite and denitrify, also increase protein percentage. *Rhizobacter* is mainly involved in the biological control of plant pathogens, nutrient cycling and seedling establishment.

The objective of this study was isolation and identification of biofertilizers from soil, root nodules and cereals, the activity of these biofertilizers were checked under the various stages of development. Biofertilizer inoculants are used to increased plant yield in several countries and commercial products are commercially available. Several biofertilizers are commercially produced and used to different crops, mostly using strains of *Azotobacter, Rhizobium, Azospirillum, Burkholderia* and *Phosphobacter* [4]. Enhance the soil conditions, balancing the pH level. The microbial biofertilizer is Eco-friendly and it does not cause environmental pollution and produce organic acids, antibiotics, Indole, acetic acid and antifungals in the root system and improve the Rhizophere microflora in the plant. Microbial biofertilizer can Significantly increases the available phosphorous in the soil which could improve the seed number in plant and micro and macro nutrient concentrations in seeds [11]. Maize leaf number, shoot dry weight, leaf surface area were significantly increases by bacterial inoculation. Similar results were obtained by [6, 2]. Using commercial Egyptian biofertilizers microbial containing nitrogen fixing bacteria and Microbial biofertilizer found that all treatments significantly increased plant growth parameters compared with control plants.

MATERIALS AND METHOD

Collection of samples and Isolation of *Azotobacter, Azosprillium, Phosphobacter,, Rhizobacter* : Isolation of micro organisms from different paddy fields, grasses, soil and root nodules samples were collected from the various sources at Dharmapuri districts of Tamilnadu, India. The *Azotobacter* was isolated from the Rhizosphere soil, collected from the soybean cultivated soils. The soil samples were serially diluted up to 10^{-7} dilution and 0.1 ml of suspension was spread on Jensen's medium and incubated at 30° C for 5 days.

Azospirillum was isolated from from the Rhizosphere soil of paddy and the collected samples were aseptically transferred to the laboratory. The soil samples were serially diluted up to 10^{-7} dilution and 0.1 ml of suspension was spread on malic acid medium and incubated at 30° C for 7 day.

P*hosphobacter* were collected from the rhizosphere Soil samples of soybean and air dried. The soil samples were serially diluted upto 10^{-7} dilution. 0.1 ml suspension was spread on Pikovskaya's medium and incubated at 28° C for 7 days. And *Rhizobacter* were collected from soybean cultivated Rhizosphere soils. And soil samples were serially diluted upto 10^{-7} dilution. Finally, 0.1 ml suspension was spread on yeast extract mannitol agar (YEMA) medium and incubated at 30° C for 5-7 days.

Microscopic and biochemical characterization of micro organisms: The bacterial isolates were identified based on the Bergey's Classification of Systematic Bacteriology.

Experiment design and inoculam treatment of seed : The field experiment was conducted during December – January (Margazhipattam) in Ariakulam in Dharmapuri. The experimental site of soil sample was sandy loam and alkaline (pH - 8.2). The selected land dimensions were 10m length and 8m breadth. The furrows and ridges were arranged in 6m long. The two types of hybrid sunflower seeds were taken. TCSH-1 and SSH-48, high quality hybrid sunflower seeds obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Biofertilizer inoculants were mixed with small quantity of water and treated with seed. In case of seed dipping inoculum suspension was prepared in the ratio of 1:10. The seeds were dipped in suspension and kept immersed for about 12 hours. The seeds were taken out from the suspension and dried on air. The remaining suspension of slurry was directly sprayed on the field.

Biofertilizer treatment level: This study of microbial biofertilizer treatment includes the two types of hybrid seed varities TCSH-1 and SSH-48 microbial biofertilizer treatment of TCSH-1 is T1-Control, T2-*Azotobacter*, T3-*Azospirillum*, T4-*Phosphobacter*, T5-*Rhizobacter* and SSH-48 treatment is S1-Control, S2-*Azotobacter*, S3-*Azospirillum*, S4-*Phosphobacte*r and S5-*Rhizobacter*.

The effect of micro organisms on growth and yield of *Helianthus annuus* : The plant height, stem diameter (at successive developmental stage), 100 seed weight (counted manually from a sample drawn randomly from each treatment) was measured from the base of the plant at ground level to the point of the attachment of the capitulam at 15, 30, 45, 60 and 75 days. After sowing and expressed in centimetres. The root was absorbing nutrients, minerals from the soil. based on the nutrients uptake, the plant growth and yield parameter was varied. The root length was measuring from tip of the root and the mean, standard deviation value was calculated and expressed in centimetres.

The collected sunflower seeds were dried on direct sunlight on 2-4 days. The seed dry weight value is supporting the oil production. Measuring the dry weight of 100 seeds was measured and the mean, standard deviation value was calculated and expressed in centimetres.

Statistical analyses

Growth and yield parameters were measured (plant weight, shoot length, root length, 100 seed weight, seed dry weight.) and analyzed by statistics (Mean, and standard deviation of least significant differences were calculated.)

RESULTS AND DISCUSSION

Collection of samples and Isolation of *Azotobacter, Azosprillium, Phosphobacter, Rhizobacter spp.*

In the present study, Microbial biofertilizers were successfully isolated and identified from various sources (Rhizosphere soil, Root nodules, Paddy etc). Morphological and biochemical characterization of isolated micro organisms data's were shown Taple-1. Four microorganisms viz., *Azotobacter, Azospirillum, Phosphobacter* and *Rhizobacter* were isolated and used as biofertilizers and effect of the application of the four microbial strains to TSCH-1 and SSH-48 Hybrid seeds.

Growth and yield parameters:

Growth and yield parameters were determined at 15, 30, 45, 60 and 75 days after sowing and expressed in statistical method. The TCSH-1 hybrid seed production shows increased plant height is 145.3 cm, similarly root length – 9.2 cm, stem diameter – 1.5 cm, Leaf size – 27.8 cm, Hundred seed weight – 10.4 g. and SSH-48 Hybrid seed production shows growth of plant height – 138.3 cm, leaf size – 15.2 cm, depth of the root length – 9.28 cm, stem diameter – 1.1 cm, then significantly increased hundred seed weight is – 8.15 g were shown Graph-1. Growth and yield parameter was measured by mean and standard deviation value. The TCSH-1 showed 45.875% increased growth and 90% increased yield when compared then SSH-48 is showed 40.95% growth and 60% increased yield. This indicates sunflower plants are more utilizing on microbial biofertilizer. Essential and more effective when compared over chemical fertilizer and control also**.**

Table-1 Microscopic and biochemical characterization of micro organisms.

S.no	Sample	Gram staining test	Shape	Motility test	Catalase test	Oxidase test	Selective medium	Probable Micro organisms
1.	Rhizosphere soils	Gram negative	bacilli	Motile	Positive	Positive	YEMA medium	*Rhizobium*
2.	Root of paddy	Gram negative	bacilli	Motile	Positive	Positive	Malic acid medium	*Azospirillum*
3.	Rhizosphere soils	Gram negative	bacilli	Motile	Negative	Positive	Jensen's medium	*Azotobacter*
4.	Rhizosphere soils	Gram negative	bacilli	Motile	Positive	Positive	Pikovskaya's medium	*Phosphobacter*

TCSH-1 and SSH-48 Hybrid seed production- plant height, leaf size, stem diameter, root length, hundred seed weight.

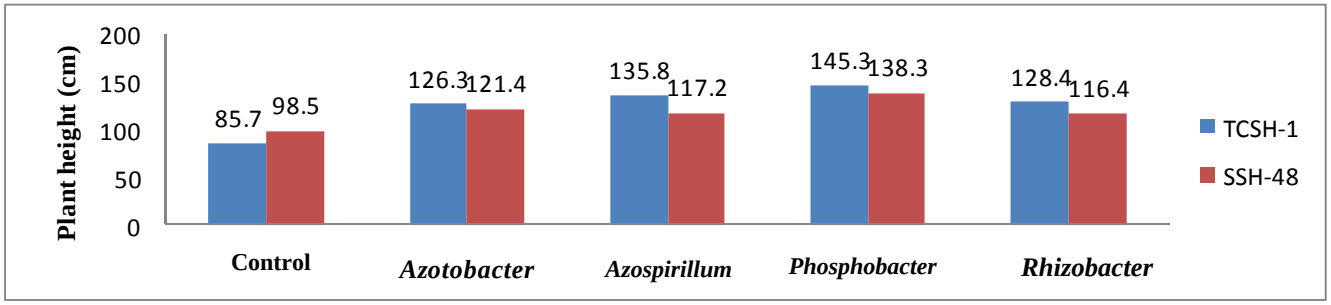

Figure 1 Plant height (cm)

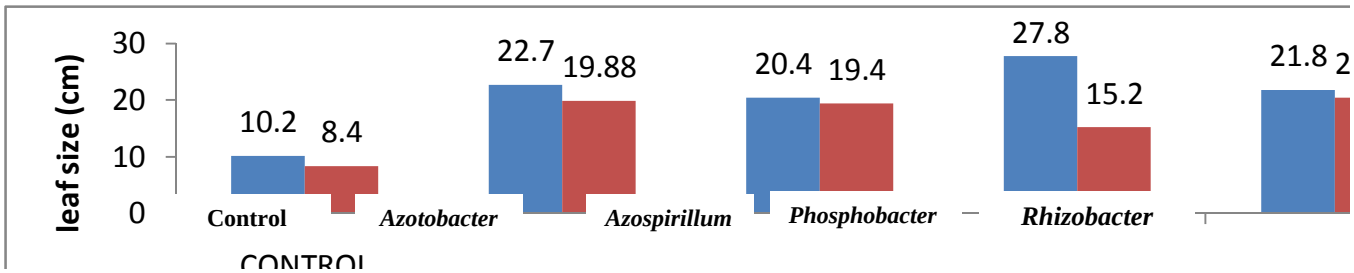

Figure 2 Leaf size (cm)

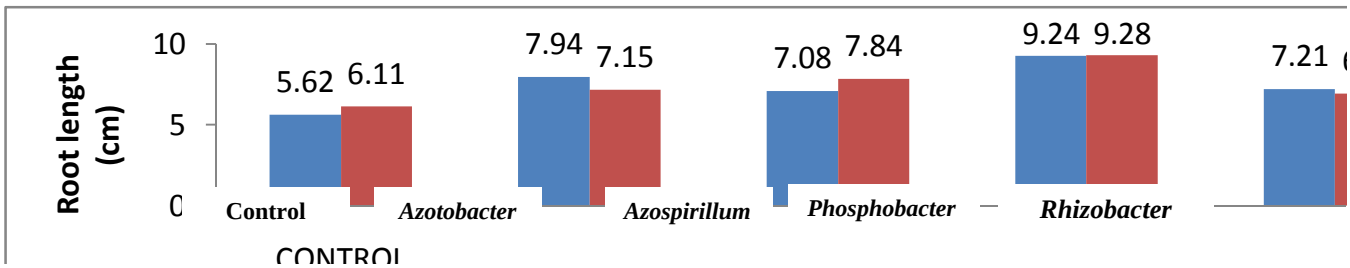

Figure 3. Root length (cm)

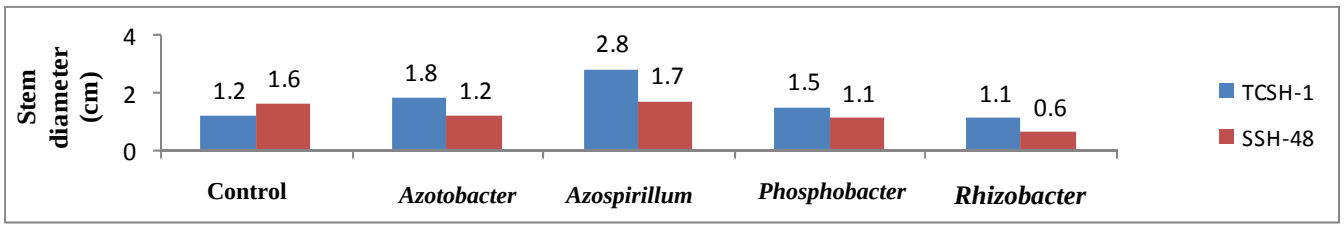

Figure 4. Stem diameter (cm)

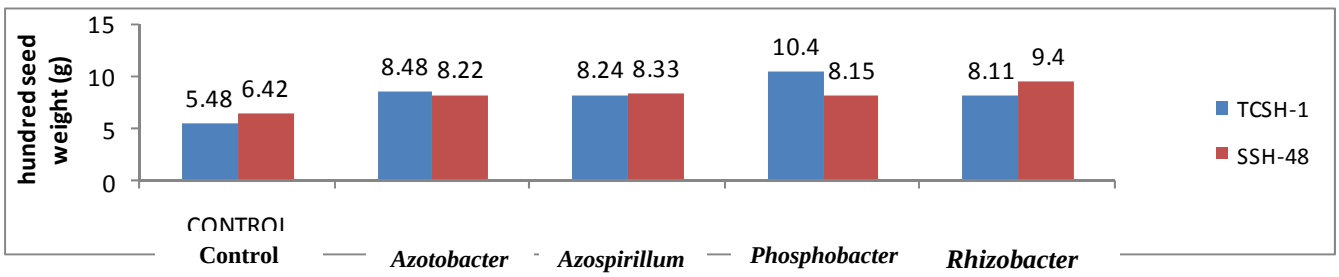

Figure.5. Hundred seed weight

CONCLUSION

Bacterial biofertilizer are symbiotically associated with cultivated crops including sunflower, maize with bacterial biofertilizer assist the plant in increasing nutrient uptake and resist root micro organisms attacking plants. The effect of bacterial inoculation on phosphorus uptake, and yield responses of sunflower in field experiment have been carried out on sunflower. Bacterial biofertilizer are commonly called as microbial inoculants which are capable of mobilizing important nutritional elements in the soil from unusable to usable form. It is considered as host for various crops, and adapts different habitats and variability in quality and quantity .It's isolation technique and mass production methods has been regarded as a boon for agriculture and restoration of disturbed eco systems. The effect of bacterial biofertilizer on yield of sunflower was investigated. Sunflower (*Helianthus annus* L.,) is an important oil seed crop in India. A positive effect of bacterial biofertilizer inoculation on growth and yield of sunflower were observed. TCSH-1 and SSH-48 variety of sunflower (*Helianthus annus* L.,) were studied and standard deviation on the effect of growth and seed yield was calculated. In average, standard deviation analysis, plant height, number of leaves, stem diameter percentage of seed filling and seed dry weight showed significantly positive average, standard deviation (TCSH-1, SSH-48). There is a lot of similarity among the results obtained by average and standard deviation.

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