International Journal of Trend in Scientific Research and Development (IJTSRD)
International Open Access Journal
ISSN No: 2456 - 6470 | www.ijtsrd.com | Volume - 2 | Issue – 3

Characterization of Soil Microbes and Viability Assessment of Liquid Microbial Consortium and Its Effect on the Growth and Yield of *Vignaradiata L.*

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ABSTRACT

The present study was carried out to isolate and identify the bacterial and fungal species from paddy field soil at Vedharaniyam, Nagappatinam District, Tamilnadu, South India. The bacterial and fungal species such as *Rhizobium*, *Azotobacter*sp and *Azospirillum*sp, *Aspergillus*sp, *Trichoderma*sp and *Penicillium*sp respectively were isolated from paddy field soil by Serial dilution agar plating method. The isolated bacterial and fungal species were prepared as liquid bacterial and fungal consortium and separate broth cultures were also prepared by using specific media. The viability count was checked by using spread plate method as in the broth test. The effectiveness of the growth of *Vignaradiata*L. was tested by using liquid biofertilizer, using different treatments. The seeds were treated with the prepared biofertilizers and sown in 10 pots of equal size. The seedlings of each pot were treated with liquid biofertilizers. The uninoculated pot was denoted as control. Then the morphological parameter such as height of the plant, number of leaves, number of flowers, shoot length, root length, number of roots , inter nodal length, leaf fresh weight, leaf dry weight, root fresh weight, root dry weight, number of seeds, number of root nodules, number of pods and yield were analyzed at different intervals (30^{th}, 45^{th} and 60^{th} days). Compared to all combined inoculation of liquid biofertilizer T4 and T9 in 60^{th} days showed better response in all the parameters tested.

Keywords: *Biofertilizer, Uninoculated, VignaradiataL. Effectiveness, Parameters, Combined*

INTRODUCTION

The mung bean is one of many species recently moved from the genus Phaseolus to *Vigna* and is still often seen cited as *Phaseolusaureus* or *Phaseolusradiatus*. These are all the same plant. Skin colour of mung bean can be classified into dark green, olivine, green black these three kinds, seed skin can be classified as lustrous and unpolished(dark green).The best grade is the one lustrous, big size round shape and easy broken when boiled. Mung Bean is a traditional food source of our Chinese people. Vitamins, calcium, irons and phosphorus ratio higher than crude rice.

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants in uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants. Very often microorganisms are not as efficient in natural surroundings as one would expect them to be and therefore, artificially multiplied cultures of efficient selected microorganisms play a vital role in accelerating the microbial processes in soil.

Biofertilizer is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable

agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function. The need for the use of biofertilzers has arisen, primarily because of two reasons. The increased usage of chemical fertilizers leads to damage in soil texture and raises other environmental problems. Therefore, the use of biofertilzers is both economical and environment friendly.

Liquid Biofertilizers

Liquid biofertilizers preparation comprises requirements to preserve organisms and deliver them to the target regions to improve their biological activity or a consortium of microorganisms provided with suitable medium to keep up their viability for certain period which aids in enhancing the biological activity of the target site. Liquid formulation is a budding technology in India and has very specific characteristics and uniqueness in its production methods. Liquid biofertilizers are the microbial preparations containing specific beneficial microorganisms which are capable of fixing or solubilizing or mobilizing plant nutrients by their biological activity.

Bacterial Biofertilizer

Many rhizospheric bacterial strains possess plant growth-promoting mechanisms. These bacteria can be applied as biofertilizers in agriculture and forestry, enhancing crop yields. Bacterial biofertilizers can improve plant growth through several different mechanisms. Several plant growth-promoting rhizobacteria (PGPR) have been used worldwide for many years as biofertilizers, contributing to increase in crop yields and soil fertility and hence, having the potential to contribute to more sustainable agriculture and forestry. The technologies for the production and application of bacterial inoculum are under constant development and improvement and the bacterial-based biofertilizer market is growing steadily.

Fungal Biofertilizers

Fungal biofertilizers comprise fungal inoculum either alone or in combination, exerting direct or indirect benefits on plant growth and crop yield through different mechanisms. Fungal biofertilizers, which have been used to improve plant growth by enhancing phosphorus absorption in plants, are phosphate solubilizing microorganisms. The commonly widespread fungi are *Penicillium, Aspergillus*and*Trichoderma*species. There are a number of biofertilizers available in the market. However, applications are based on their ability to supply and mobilize plant nutrients, control plant diseases and promote plant growth and development.

The mung bean or green gram is one of many species recently moved from the genus Phaseolus to *Vigna* and is still often seen cited as *Phaseolusaureus* or *Phaseolusradiatus*. These are all the same plant. Skin colour of mung bean can be classified into dark green, olivine, green black these three kinds, seed skin can be classified as lustrous and unpolished(dark green). The best grade is the one lustrous, big size round shape and easy broken when boiled. Mung Bean is a traditional food source of our Chinese people. Vitamins, calcium, irons and phosphorus ratio higher than crude rice.

MATERIALS AND METHODS

Soil samples were collected from paddy field at Vedharaniyam, Nagappatinam District, Tamilnadu, South India.Soil samples were taken from each container and subjected to serial dilution followed by pour plate method. Bacterial species were identified by Gram's staining, motility and biochemical tests. Fungal species were identify the by Lacto phenol cotton blue staining.Identified bacterial species such as *Rhizobium*sp, *Azospirillum*sp, *Azotobacter*spand fungal species *Penicillium*sp, *Aspergillus*spand *Trichoderma* sp.

Preparation of Bacterial and Fungal Liquid Biofertilizer

Prepared bacterial and fungal starter culture byspecific medium. Nutrient broth was used for bacteria and Rose Bengal broth was used for fungi.50ml broth of all three bacteria *Rhizobium*sp, *Azotobacter*sp and *Azospirillum*sp as a liquid bio-fertilizer was prepared.Three broths were mixed and shakevigorously; this mixture was again incubated for 2 days. Now this broth was called liquid bacterial consortium. 50ml broth of all three fungi *Penicillium*sp, *Aspergillus*spand *Trichoderma*sp as a liquid bio-fertilizer was prepared. Three broths were mixed and shake vigorously; this mixture was again incubated for 10 days. Now this broth was called liquid fungal consortium.

Confirmatory test for bacteria

Confirmatory test were done to identify the presence or absence of specific bacteria in the liquid bacterial consortium.

Confirmatory Test for *Rhizobium* s

LactoseAgar Test

Rhizobium spwas spread out on agar medium containing lactose (10 g\1). The plates were flooded with Benedict's reagent after 4-10 days. The growth of *Rhizobium*spin this medium was absent. This indicated the confirmation of *Rhizobium* sp.

Confirmatory Test for *Azospirillum*sp

Pellicle Test

The active *Azospirillum*spisolates were inoculated at subsurface level in screw cap tubes containing sterilized semisolid N- free malate medium (Okon*et al*., 1977) under aseptic conditions. The tubes were incubated at 30°C for a period of one week and observed for growth of *Azospirillum*spas subsurface pellicle.

Confirmatory Test for *Azotobacter*sp

Cyst formation

*Azotobacter*sp have ability to form cysts under adverse conditions. Presence of cyst is as one of the criterion for identification of these isolates. The *Azotobacter*spisolates were grown N-free agar medium for 7 days. These isolates were stained with a mixture of neutral red and light green SF yellowish, observed under oil immersion microscope.

Mass Production of Liquid Biofertilizer

Theisolatedstains were grown in respective broth medium in culture tube. After checking the culture for purity and proper growth, the culture was transferred from culture tube to small conical flask containing sterilized liquid medium as starter culture. Later the starter culture was transferred to a large conical flask on a rotary shaker at 150 rpm for 5 days at 28±2ºC.

Viability Count

The number of living cells was counted by spread plate method. Doing spread plate by making serial dilutions from 10^{-1} to 10^{-7} (depend on concentrations) then the replicates of 0.1 ml of broth from 10^{-6} and 10^{-5} was spread over the nutrient agar plates. The plates were incubated in incubator at 37ºC for 7 days. The number of cells(ml) present in 0.1 ml of broth was determined by multiplying total number of colonies with dilution factor.

$$\text{No of cells\ml} = \frac{\text{Mean no of colonies}}{\text{Volume of inoculum}} \times \text{dilution factor}$$

Testing the Efficiency of liquid biofertilizer

Pot Culture

The efficiency of liquid biofertilizers on the growth and yield of *Vignaradiata*L.was studied using 8 different bacterial and fungal liquid formulations and an uninoculated control for each also maintained.

The bacterial liquid formulation treatments were,

T1 – *Rhizobium* sp
T2 – *Azospirillum*sp
T3 – *Azotobacter*sp
T4 – *Rhizobium* sp + *Azospirillum*sp + *Azotobacter*sp
T5 – Control

The fungal liquid formulation treatments were,

T6 – *Aspergillus*sp
T7 – *Trichoderma*sp
T8 – *Penicillium*sp
T9 – *Aspergillus*sp + *Trichoderma*sp + *Penicillium*sp
T10 – Control

The seeds were treated with the prepared biofertilizers and sown in 10 pots of equal size. The seedlings of each pot were treated with liquid biofertilizers. The uninoculated pot was denoted as control. Liquid biofertilizer was sprayed on plants at 10 days intervals.

The morphometric parameters height of the plant (in cm), number of leaves (per plant), number of flowers (per plant), shoot length (in cm), root length (in cm), number of roots (per plant), inter nodal length (in cm), leaf fresh weight (mg\plant), leaf dry weight (mg\plant) , root fresh weight (mg\plant), root dry weight (mg\plant), number of seeds (in plant), number of root nodules (per plant), number of pods (per plant) and yield (seed in gram) was measured at 30th, 45th and 60th days of growth.

Statistical Analysis (Gupta, 2004)

All the experiment was repeated as triplicates. The result obtained in the present study was subjected to

statistical analysis such as Mean (X) and Standard Deviation (SD).

Mean (X) $= \frac{\Sigma X}{N}$

Where,

Mean (X) – Sum of all values of the variable

N – Number of observation.

Where, add together all values of variable X and obtain X- X. Divide the total by the number of observation.

The standard deviation calculated by the formula,

$$S.D = \frac{\sqrt{\Sigma(X - \bar{X})^2}}{n-1}$$

Where, $\bar{X}$ - Arithmetic mean, X – Number of all values and N- Total number of observation. Find out the deviation of each value from the mean (X- $\bar{X}$) square the deviation and take the total of square deviation. Divide the total number of observation.

Table:1 Morphological and Biochemical Characteristics of Isolated Bacteria

S.No	Characteristics	*Rhizobium* sp	*Azospirillum*sp	*Azotobacter*sp
Morphological Characteristics				
1.	Gram Staining	–	–	+
2.	Motility	Motile	Motile	Motile
3.	Shape	Rod	Rod	Spherical
Biochemical Characteristics				
4.	Indole	+	–	+
5.	MR	+	–	+
6.	VP	–	–	–
7.	Citrate	–	–	+
8.	Catalase	+	+	+
9.	Triple Sugar Iron	+	+	+
10.	Carbohydrate Fermentation	–	+	+

(+) – Positive ,**(-)** – Negative

Table : 2 Colonial and Morphological Characteristics of IsolatedFungi

S. No	Organisms	Colony Morphology	Microscopic Observation
1.	*Aspergillus*sp	Blackish brown	Hyphae septate with conidiospore
2.	*Penicillium*sp	Bluish green to clear green	Aerial hyphae with conidiospore
3.	*Trichoderma*sp	White to pink	Two celled conidia

Table : 3 Details of Viability Count of bacteria (CFU\ml)

Species	Storage time (in months)			
	0	1	2	3
Rhizobium sp	1.9×10^7	2.3×10^6	2.5×10^5	1.8×10^5
Azospirillumsp	1.5×10^6	1.25×10^6	3×10^5	1.9×10^5
Azotobactersp	1.7×10^7	3×10^8	2×10^6	1.2×10^5

Table: 4 Details of Viability Count of fungi (CFU\ml)

Species	Storage time (in months)			
	0	1	2	3
Aspergillussp	2×10^7	1.3×10^6	3.5×10^5	2.8×10^5
Penicilliumsp	1.2×10^6	2.5×10^7	1.9×10^6	3.4×10^5
Trichodermasp	1.5×10^7	1.9×10^7	1.7×10^6	2.5×10^5

Table: 5 Effect of liquid biofertilizer on morphological parameters of *Vignaradiata*L . (30th day)

Morphological Parameters	Treatments									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Height of the plant (in cm)	10±5.1	9±3.3	9±4.5	4±2.3	8±2.2	9±2.2	8±3.4	9±2.5	13±2.5	7±2.2
Inter nodal length (in cm)	4±5.1	4±3.4	4±4.2	5±4.2	3±2.1	4±2.5	3±9.2	4±3.2	5±2.5	3±2.3
Number of leaves (per plant)	9±2.1	9±0.2	9±1.5	9±4.5	7±1.2	8±3.5	8±1.2	8±4.5	9±3.2	7±2.3
Leaf fresh weight (mg\plant)	8±7.5	8±5.7	8±6.8	8±9.5	6±2.5	7±2.5	7±1.2	7±5.9	8±8.2	6±5.2
Leaf dry weight (mg\plant)	6±6.5	6±4.1	6±5.5	6±8.5	5±1.5	6±3.2	6±1.5	6±2.8	6±7.2	5±4.9
Number of root nodules (per plant)	6±4.5	5±2.6	5±2.8	7±6.5	4±1.2	5±2.5	4±2.5	5±2.6	7±5.7	4±2.2
Shoot length (in cm)	7±1.4	4±9.5	5±1.5	7±2.1	3±1.5	4±4.2	4±1.5	4±9.5	7±1.4	3±3.8
Root length (in cm)	5±4.5	5±3.5	5±4.2	7±1.2	4±1.5	5±3.5	4±4.5	5±4.2	6±4.5	4±3.5
Root fresh weight (mg\plant)	8±8.5	7±4.5	8±2.3	9±9.5	5±1.5	6±4.9	6±1.3	7±1.5	9±1.5	5±4.9
Root dry weight (mg\plant)	7±2.5	6±5.4	7±1.2	7±4.2	5±2.2	6±3.2	6±2.1	6±4.1	7±3.8	5±3.5

Table :6 Effect of liquid biofertilizer on morphological parameters of *Vignaradiata*L. (45th day)

Morphological Parameters	Treatments									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Height of the plant (in cm)	12±5.1	11±2.2	11±0.5	16±2.4	8±2.7	9±4.5	9±3.5	10±2.1	15±2.6	8±8.2
Inter nodal length (in cm)	6±4.2	5±2.5	6±2.9	7±4.5	4±2.9	5±3.7	4±3.6	5±8.4	6±5.8	4±9.5
Number of leaves (per plant)	11±2.3	10±4.9	11±1.5	12±2.3	8±4.9	9±5.2	9±3.5	10±2.5	11±4.9	8±2.8
Leaf fresh weight (mg\plant)	11±3.9	11±1.2	11±2.2	12±2.5	7±1.5	9±2.5	9±1.5	10±9.2	12±1.2	7±5.5
Leaf dry weight (mg\plant)	8±3.8	7±4.9	8±2.5	9±5.6	5±2.5	6±7.2	6±2.2	7±5.6	9±4.9	5±5.9
Number of root nodules (per plant)	7±4.7	6±8.9	7±2.7	8±6.7	5±1.5	6±2.4	6±0.6	6±8.8	8±5.9	5±4.2
Shoot length (in cm)	7±3.2	6±2.5	5±2.8	8±1.2	3±6.8	4±8.5	4±4.5	5±0.2	7±8.2	3±8.2
Root length (in cm)	6±4.2	6±0.8	6±1.5	8±5.7	4±4.3	5±6.8	5±5.5	5±7.5	8±4.5	4±5.3
Root fresh weight (mg\plant)	10±2.5	9±2.3	9±4.5	10±5.6	6±4.2	8±2.6	7±4.9	8±4.5	10±4.5	7±3.1
Root dry weight (mg\plant)	9±1.5	8±1.2	8±2.6	9±4.5	6±3.1	7±3.2	7±1.5	7±4.9	9±3.2	6±4.2
Number of flowers (per plant)	12±1.5	11±6.4	11±7.5	16±2.3	7±7.3	10±6.5	10±4.7	9±5.2	15±2.7	8±4.3

Table – 7 Effect of liquid biofertilizer on morphological parameters and yield of *Vignaradiata*L. (60th day)

Morphological Parameters	Treatments									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Height of the plant (in cm)	23±2.9	21±3.9	22±4.1	25±2.9	16±8.2	20±3.5	19±5.1	21±2.6	24±8.7	17±1.9
Inter nodal length (in cm)	9±4.3	8±4.5	7±2.5	10±4.5	6±1.5	7±2.3	7±1.5	7±5.6	10±1.5	6±4.9
Number of leaves (per plant)	19±2.8	17±2.6	18±2.9	21±5.5	9±9.8	16±2.8	16±2.5	16±4.7	20±8.7	10±1.2

Leaf fresh weight (mg\plant)	14±5.2	14±3.2	14±4.5	15±4.5	11±1.5	13±5.6	12±2.5	14±2.3	15±1.5	11±3.2
Leaf dry weight (mg\plant)	13±3.2	12±4.5	13±1.2	13±5.2	10±3.5	11±4.9	11±1.5	12±3.5	13±4.5	10±4.2
Number of pods (per plant)	12±1.5	11±4.5	11±6.5	14±2.3	8±2.2	10±1.6	9±4.6	10±5.5	13±2.5	8±3.5
Number of seeds (per plant)	16±4.6	16±0.2	16±1.5	19±9.5	12±0.9	14±2.5	13±2.8	15±2.5	17±8.9	12±3.2
Number of root nodules (per plant)	19±4.5	18±4.2	18±4.8	20±8.5	16±2.5	17±4.5	17±2.8	17±5.6	19±7.9	16±4.6
Shoot length (in cm)	11±6.7	10±1.5	9±4.2	13±4.1	7±2.5	8±4.5	8±1.2	9±2.5	12±4.2	7±4.5
Root length (in cm)	11±4.9	11±1.2	11±3.4	12±4.5	9±2.5	10±3.9	10±2.5	10±4.6	12±1.5	9±4.5
Root fresh weight (mg\plant)	12±4.5	11±4.8	12±2.5	13±2.5	8±3.5	10±3.2	9±1.9	10±4.5	13±0.2	8±4.2
Root dry weight (mg\plant)	9±6.7	9±1.5	9±4.2	10±2.5	6±4.5	8±4.5	8±2.2	8±5.6	10±0.7	7±2.1
Yield (seed in gram)	16±4.6	16±0.2	16±1.5	19±9.5	12±0.9	14±2.5	13±2.8	15±2.5	17±8.9	12±3.2

Values are triplicates, mean ± standard deviation

RESULTS AND DISCUSSION

The present study was carried out to isolate and identify the bacterial and fungal species from paddy field soils at Vedharaniyam, Nagappatinam District, Tamilnadu, South India. The effect of different liquid biofertilizer on growth and productivity of *Vignaradiata*L. were studied. The results shown that viability of bacterium and fungi tend to decline during storage of biofertilizer but did not significantly reduce the effect on growth and production of plant. Generally, fungi and bacteria found in deep layer or slow growing due to unavailability of mineral nutrients and compaction of soil along depth (Dkhar and Mishra, 1992).

Physical features of liquid *Rhizobium*spwasdull white in colour, No bad smell, No foam formation and pH 6.8 to 7.5 was observed. Colour of the liquid *Azospirillum*sp may be blue or dull white. Bad odours confirm improper liquid may be broth. Production of yellow gummy colour materials confirms the quality product. Acidic pH always confirms no *Azospirillum*spbacteria present in liquid (Pindi and Satyanarayana, 2012).

Morphometric Parameters

Height of the Plant (in cm)

At 30th day, maximum height of the plant was recorded in T4 (14±2.3) and T9 (13±2.5) the combined inoculations, followed by other treatments, T1 (10±5.1), T3 (9.2±4.5), T2 (9±3.3), T8 (9±2.5), T6 (9±2.2), T7 (8±3.4), T10 (7±2.2) and T5 (8±2.2). On 45th day, maximum height of the plant was observed in combined inoculation T4 (16±2.4) and T9 (15±2.6) followed by T1 (12±5.1), T3 (11±0.5), T2 (11±2.2), T8 (10±2.1), T6 (9±4.5), T7 (9±3.5), T10 (8±8.2) and T5 (8±2.7). On 60th day, maximum height of the plant was shown by T4 (25±2.9) and T9 (24±8.7) (21±2.6) followed by T1 (23±2.9), T3 (22±4.1), T2 (21±3.9), T8 (21±2.6), T6 (20±3.5), T7 (v), T10 (17±1.9) and T5 (16±8.2) (Plate – VII, Fig - 1 and Table -5 to 7).

Number of Leaves (per plant)

At 30th day, maximum number of leaves in the plant was recorded in T4 (9±4.5) and T9 (9±3.2) the combined inoculations, followed by T1 (9±2.1), T3 (9±1.5), T2 (9±0.2), T8 (8±4.5), T6 (8±3.5), T7 (8±1.2), T10 (7±2.3) and T5 (7±1.2). On 45th day, maximum number of leaves in the plant was observed in combined inoculation of T4 (12±2.3) and T9 (11±4.9) followed by T1 (11±2.3), T3 (11±1.5), T2 (10±4.9), T8 (9±5.2), T6 (9±3.5), T7 (10±2.5), T10 (8±2.8) and T5 (8±4.9). On 60th day, maximum leaves in the plant was shown by T4 (21±5.5) and T9 (20±8.7) followed by T1 (19±2.8), T3 (18±2.9), T2 (17±2.6), T8 (16±4.7), T6 (16±2.8), T7 (16±2.5), T10 (10±1.2) and T5 (9±9.8) (Table – 5 to 7).

Number of Flowers (per plant)

On 45th day maximum number of flowers in the plant was recorded in T4 (16±2.3) and T9 (15±2.7) followed by other liquid biofertilizer treatments T1 (12±1.5), T3 (11±7.5), T2 (11±6.4), T8 (9±5.2), T6 (10±6.5), T7 (10±4.7), T10 (8±4.3) and T5 (7±7.3) (Table - 6).

Number of Root Nodules (per plant)

Among the overall treatments on 30th day, maximum number of root nodules were recorded in combined inoculation such as, T4 (7±6.5) and T9 (7±5.7) followed by T1 (6±4.5), T3 (5±2.8), T2 (5±2.6), T8 (5±2.6), T6 (5±2.5), T7 (4±2.5), T10 (4±2.2) and T5 (4±1.2). Among the overall treatments on 45th day, maximum number of root nodules were recorded in combined inoculation such as T4 (8±6.7) and T9 (8±5.9) followed by T1 (7±4.7), T3 (7±2.7), T2 (6±8.9), T8 (6±8.8), T6 (6±2.4), T7 (6±0.6), T10 (5±4.2) and T5 (5±1.5). At 60th day, maximum number of root nodules were recorded in combined inoculation such as T4 (20±8.5) and T9 (19±7.9) followed by T1 (19±4. 5), T3 (18±4.8), T2 (18±4.2), T8 (17±5.6), T6 (17±4.5), T7 (17±2.8), T10 (16±4.6) and T5 (16±2.5) (Table – 5 to 7).

Shoot Length (in cm)

On 30th day, maximum number of shoot length in the plant was observed in combined inoculation of T4 (7±1.4) and T9 (6±5.4) followed by T1 (4±9.5), T3 (5±1.5), T2 (4±9.5), T8 (4±1.5), T6 (3±1.5), T7 (4±4.2), T10 (3±3.8) and T5 (7±2.1). At 45th day, maximum number of shoot length was recorded in combined inoculation such as T4 (8±1.2) and T9 (3±8.2) followed by T1 (7±3.2), T3 (5±2.8), T2 (6±2.5), T8 (5±0.2), T6 (4±8.5), T7 (4±4.5), T10 () and T5 (3±6.8). At60th day, maximum number of shoot length was recorded in combined inoculation such as, T4 (13±4.1) and T9 (12±4.2) followed by T1 (11±6.7), T3 (9±4.2), T2 (10±1.5), T8 (8±1.2), T6 (8±4.5), T7 (8±1.2), T10 (7±4.5) and T5 (7±2.5) (Table – 5 to 7).

Root Length (in cm)

On 30th day, maximum number of root length in the plant was observed in combined inoculation of T4 (7±1.2) and T9 (6±4.5) followed by T1 (5±4.5), T3 (5±4.2), T2 (5±3.5), T8 (5±4.2), T6 (5±3.5), T7 (4±4.5), T10 (4±3.5) and T5 (4±1.5) (Table – 5 to 7). At 45th day, maximum number of root length in the plant was observed in combined inoculation of T4 (8±5.7) and T9 (8±4.5) followed by T1 (6±4.2), T3 (6±1.5), T2 (6±0.8), T8 (5±7.5), T6 (5±6.8), T7 (5±5.5), T10 (4±5.3) and T5 (4±4.3). AtIn 60th day, maximum number of root length in the plant was observed in combined inoculation of T4 (12±4.5) and T9 (12±1.5) followed by T1 (11±4.9), T3 (11±3.4), T2 (11±1.2), T8 (10±4.6), T6 (10±3.9), T7 (10±2.5), T10 (9±4.5) and T5 (9±2.5).

Internodal Length (in cm)

At 30th day, maximum level of inter nodule length was recorded in combined inoculations, i.e., T4 (5±4.2) and T9 (5±2.5) followed by T1 (4±5.1), T3 (4±4.2), T2 (4±3.4), T8 (4±3.2), T6 (4±2.5), T7 (3±9.2), T10 (3±2.3) and T5 (3±2.1). On 45th day, maximum level of inter nodule length was recorded in combined inoculations, T4 (7±4.5) and T9 (6±5.8) followed by T1 (6±4.2), T3 (6±2.9), T2 (5±2.5), T8 (5±8.4), T6 (5±3.7), T7 (4±3.6), T10 (4 ±9.5) and T5 (4±2.9). At 60th day, maximum level of inter nodule length was recorded in combined inoculations, T4 (10±4.5) and T9 (10±1.5) followed by T1 (9±4.3), T3 (7±2.5), T2 (8±4.5), T8 (7±5.6), T6 (7±2.3), T7 (7±1.5), T10 (6±4.9) and T5 (6±1.5) (Fig – 1 and Table – 5 to 7).

Leaf Fresh Weight (mg\pant)

At 30th day, maximum level of leaf fresh weight was recorded in combined inoculations, T4 (8±9.5) and T9 (8±8.2) followed by other treatments, T1 (8±7.5), T3 (8±6.8), T2 (8±5.7), T8 (7±5.9), T6 (7±2.5), T7 (7±1.2), T10 (6±5.2) and T5 (6±2.5). At 45th day,

maximum level of leaf fresh weight was recorded in T4 (12±2.5) and T9 (12±1.2) the combined inoculations, followed by T1 (11±3.9), T3 (11±2.2), T2 (11±1.2), T8 (10±9.2), T6 (9±2.5), T7 (9±1.5), T10 (7±5.5) and T5 (7±1.5). At 60th day, maximum level of leaf fresh weight was observed in combined inoculation of T4 (15±4.5) and T9 (15±1.5) followed by T1 (14±5.2), T3 (14±4.5), T2 (14±3.2), T8 (14±2.3), T6 (13±5.6), T7 (12±2.5), T10 (11±3.2) and T5 (11±1.5) (Table – 5 to 7 and Fig - 3).

Leaf Dry Weight (mg\plant)

At 30th day, maximum level of leaf dry weight was observed in combined inoculation of T4 (6±8.5) and T9 (6±7.2) followed by other treatments T1 (6±6.5), T3 (6±5.5), T2 (6±4.1), T8 (6±2.8), T6 (6±3.2), T7 (6±1.5), T10 (5±4.9) and T5 (5±1.5). On 45th day, maximum level of leaf dry weight was shown by combined inoculation of T4 (9±5.6) and T9 (9±4.9) followed by other treatments, T1 (8±3.8), T3 (8±2.5), T2 (7±4.9), T8 (7±5.6), T6 (6±7.2), T7 (6±2.2), T10 (5±5.9) and T5 (5±2.5). In 60th day maximum level of leaf dry weight was shown by combined inoculation of T4 (13±5.2) and T9 (13±4.5) followed by T1 (13±3.2), T3 (13±1.2), T2 (12±4.5), T8 (12±3.5), T6 (11±4.9), T7 (11±1.5), T10 (10±4.2) and T5 (10±3.5) (Table – 5 to 7 and Fig - 3).

Root Fresh Weight (mg\plant)

At 30th maximum level of root fresh weight was observed in combined inoculation of T4 (9±9.5) and T9 (9±1.5) followed by T1 (8±8.5), T3 (8±2.3), T2 (7±4.5), T8 (7±1.5), T6 (6±4.9), T7 (6±1.3), T10 (5±4.9) and T5 (5±1.5).On 45th day, maximum level of root fresh weight was shown by combined inoculation of T4 (10±5.6) and T9 (10±4.5) followed by T1 (10±2.5), T3 (9±4.5), T2 (9±2.3), T8 (8±4.5), T6 (8±2.6), T7 (7±4.9), T10 (7±3.1) and T5 (6±4.2). On 60th day, maximum of root fresh weight was recorded in combined inoculation of T4 (10±2.5) and T9 (10±0.7) followed by other treatments T1 (9±6.7), T3 (9±4.2), T2 (9±1.5), T8 (8±5.6), T6 (8±4.5), T7 (8±2.2), T10 (7±2.1) and T5 (6±4.5) (Fig – 5 and Table – 5 to 7).

Root Dry Weight (mg\plant)

At 30th day, maximum level of root dry weight was observed in T4 (7±4.2) and T9 (7±3.8) followed by other treatments T1 (7±2.5), T3 (7±1.2), T2 (6±5.4), T8 (6±4.1), T6 (6±3.2), T7 (6±2.1), T10 (5±3.5) and T5 (5±2.2). At 45th day, maximum level of root dry weight was observed in T4 (9±4.5) and T9 (9±3.2) followed by other treatments T1 (9±1.5), T3 (8±2.6), T2 (8±1.2), T8 (7±4.9), T6 (7±3.2), T7 (7±1.5), T10 (6±4.2) and T5 (6±3.1). At 60th day, maximum level of root dry weight was observed in T4 (10±2.5) and T9 (10±0.7) the combined inoculations, followed by other treatments T1 (9±6.7), T3 (9±4.2), T2 (9±1.5), T8 (8±5.6), T6 (8±4.5), T7 (8±2.2), T10 (7±2.1) and T5 (6±4.5) (Fig – 5 and Table- 1 to 7).

Number of Seeds (gm.\plant)

On 60th day, maximum level of seeds were observed in T4 (19±9.5) and T9 (17±8.9) followed by other treatments T1 (16±4.6), T3 (16±1.5), T2 (16±0.2), T8 (15±2.5), T6 (14±2.5), T7 (13±2.8), T10 (12±3.2) and T5 (12±0.9).

Number of Pods (per plant)

In 60th day, maximum level of pods were observed in T4 (14±2.3) and T9 (13 ± 2.5) the combined inoculations, followed by other treatments T1 (12±1.5), T3 (11±6.5), T2 (11±4.5), T8 (10±5.5), T6 (10±1.6), T7 (9±4.6), T10 (8±3.5) and T5 (8±2.2). (Plate - IX and Table -7)

Yield (seed in gram)

In 60th day, maximum level of yield was observed in combined inoculation of treatments such as, T4 (19 ± 9.5) and T9 (17 ± 8.9) followed by other treatments T1 (16 ± 4.6), T3 (16± 1.5), T2 (16 ± 1.2), T8 (15± 5.8), T6 (15± 2.5), T7 (13± 2.8), T10 (12 ± 3.2) and T5 (12 ± 0.9).

CONCLUSION

Bacterial and fungal biofertilizers are presently used on a very small scale as compared to chemical compounds. There has been little investment in the research and development of bacterial and fungal products because these may have poor effect in the field. Future research therefore must develop bacterial and fungal products, which have significant effect in field applications and are stable under storage.

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