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Evaluation of selected bacterial consortium formulation on the enhanced growth of *Vigna mungo* L

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Abstract
Bio fertilizer are commonly called microbial inoculants which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form by crop plants through their biological process. Liquid bio fertilizer is increasingly available in the market as one of the alternatives to chemical fertilizer and pesticide. The present paper deals with the effectiveness of the growth of *Vigna mungo* L. using liquid bio fertilizers, such as *Azospirillum* sp., *Rhizobium sp.,* and *Azotobacter* sp., with control. Shelf life of liquid bio fertilizer was maintained. The seedlings of *V.mungo* were transplanted in field which were noted as Treatment (T1-T5). The seedlings of field were treated with liquid bio fertilizers. The un-inoculated plants were denoted as control. Liquid bio fertilizer was sprayed on plants at 10 days intervals. Then morphological parameters such as leaves number, height, shoot length, root length, root nodules, yield were analyzed at different time intervals (15th, 30th, 45th & 60th days) respectively. Compare to all plants the combined inoculation of liquid bio fertilizers (T4) *Azospirillum* + *Rhizobium* + *Azotobacter* in 60th day showed better response in all the parameters was tested. To prevent the environment pollution from extensive application of chemical fertilizers the liquid bio fertilizer could be recommended to farmers to insure the public health and a substainable agriculture.

Keywords: *Azospirillum* sp., *Rhizobium sp., Azotobacter* sp, *Vigna mungo* L, liquid biofertilizers

Introduction

Bio fertilizers are one of the best modern tools for agriculture. It is a gift of our modern agricultural science. Bio fertilizers are applied in the agricultural field as a replacement of our conventional fertilizers that contains compost, household wastes and green manure. Those are not as effective as chemical fertilizers, so farmers often try to use chemical fertilizers in the agricultural field for crop improvement. But obviously the chemical fertilizers are not environment friendly. Bio fertilizers contain microorganisms which promote the adequate supply of nutrients to the host plants to ensure their proper development of growth and regulation in their physiology. Living microorganisms are used in the preparation of bio fertilizers.

Bio-fertilizers manufactured in India are mostly carrier based (solid) bio-fertilizers; the microorganisms have a shelf life of only six months. They are not tolerant of UV rays and temperatures above 30ºC. At the time of production, the population density of these microorganisms is only 108 (10 million) CFU / mL. This count reduces day by day. In the fourth month, it reduces to 10^6 (10 lakhs) cfu/ml and at the end of 6 months the count is almost nil. That's why the carrier-based bio-fertilizers were not effective and did not become popular among the farmers. These defects are rectified and fulfilled in the case of Liquid bio-fertilizers.

Liquid bio fertilizer formulation is the promising and updated technology which in spite of many advantages over the agrochemicals left a considerable dispute among the farmer community in terms of several reasons major being the viability of the organisms. Shelf life is the first and foremost problem of the carrier based bio fertilizer which is up to 3 months and it does not retain throughout the crop cycle. LBF on the other hand facilities the long survival of the organisms by providing the suitable medium which is sufficient for the entire crop cycle.

Bio fertilizer or microbial inoculants can be generally defined as latent cells of efficient strains of a phosphate solubilizing and nitrogen fixing microorganisms used for treatment of soil. Bio fertilizer are organic products of living cells containing different types of microorganisms, which have the ability to convert important elements from unavailable sources to available sources through ecological processes (Vessey, 2003) [16]. They are composting the area with the objective of increasing the number of such microorganisms and accelerate microbial process to augment to extent of the availability of the nutrient in a form which can easily assimilated by plant (Subba Rao, 1986) [15].

Materials and Methods

Collection of sample

The root nodules and soil was collected from herbal garden, S.T.E.T Women's college, sundarakottai, Thiruvarur District. Soil samples were made at a depth within 15 cm from the surface of soil. The collected sample were brought to the microbiology laboratory in sterilized polythene bags and stored in containers for further investigation.

Isolation and identification of microorganisms (Somasegaran 1994) [14]

The root nodules are kept immersed in 0.1% potassium chloride solution for 5 minutes to sterilize the surface of the nodules. The sterilized root nodules are then washed 5 or 6 times with distilled water. They are once again sterilized by immersing them in 90% ethyl alcohol for 10 seconds and washed repeatedly with distilled water. The root nodules are crushed gently in a small amount of distilled water using a pestle and mortar to get a suspension. The suspension is

diluted and inoculated on to YEMA (Yeast Extract Mannitol Agar) medium in petri dishes. The culture plates are incubated at 28˚c for about 10 days. Rhizobial cells form gummy colonies on the medium. Soil sample was subjected to serial dilution (Ronald Atlas., 1998) by pour plate method (Warcup,1950).The isolated organisms were identified by using morphological, biochemical test (Norris and ribbons., 1972) [11] using Bergey's Manual of Determinative Bacteriology (1957).

Preparation of microbial liquid fertilizer

50 ml broth of all bacteria *Azospirillum* sp., *Rhizobium sp.,* and *Azotobacter* sp., as a liquid bio fertilizer was prepared and the isolated colonies were inoculated on respective broth and incubated at 37˚c for 6-7 days. A similar procedure was followed for preparing 200ml. Then next step the broth was prepared for individual microorganism inoculated with the respective microorganism and incubated at 37˚c for 6-7 days after that mix the broths and shake vigorously, this mixture was again incubated for 2 days. Now this broth was ready to use for liquid bio Fertilizer.

Detect the efficiency of liquid fertilizer (R.P. Singh., 2002)

The consortium efficiency was detected by apply the plant *Vigna mungo* using the culture and consortium following treatment.
T1 = *Rhizobium* only
T2 = *Azospirillum* only
T3 = *Azotobacter* only
T4 = *Rhizobium* + *Azospirillum*+ *Azotobacter* Consortium
T5 = Control

Sampling method

The plant samples were collected once in 15 days for the measurement of various growth parameters from each row of field, 2 plants were randomly selected. The plant samples were washed with running water carefully.

Analysis of plant growth parameter

The morphometric parameters were studied at 15th, 30th, 45th and 60th days of growth. The study was run for 60 days. After that, the plants were randomly uprooted for analysis of plant growth parameters.

- Height of the plant (in cm)
- Number of leaves (per plant)
- Number of flowers (per plant)
- Root length (in cm)
- Number of seeds (per plant)
- Number of root nodules (per plant)
- Number of pods (per plant)
- Yield (seed in gram)

Estimation of biochemical constituents

Carbohydrate, protein and total chlorophyll content were also estimated.

Estimation of carbohydrate

Phenol sulphuric acid method as described by Roberts *et al.,* 2011 was used to estimate carbohydrate contents in selected plant. Powder of each seeds (0.1g) was mixed in 5mL of 2.5 N-HCl and heated in water bath for 3 hours. It was then neutralized by adding sodium carbonate and volume was increased up to 100ml. Glucose was used as standard for carbohydrate estimation. Each experimental sample (standard and powdered seeds) was mixed with 1 ml of 5% phenol solution and 5ml of 90% sulphuric acid solution and kept at 30˚C for 20 min., after that absorbance was taken at 490 nm and linear regression equation obtained from standard curve was used to estimate carbohydrate in plant seed

Extraction of protein (Regnar Flengsrud, 2019)

The leaves were cut into 0.5 to 1.0 cm parts and needles into 2-4 mm parts. Typically, start with 0.4g green tissue and grind it twice in a mortar with liquid N2 to give a fine powder. The storage of plant material prior to homogenization should be considered. Add an amount of insoluble polyvinylpyrrolidone (Polyclar AT) twice the weight of plant material and mix. Extraction buffer were added (9.5ml) to the mixture in the mortar, stir for a few minutes and centrifuge at +5ºC for 40 min at 8000g. Transfer the supernatant to a thick-walled glass centrifuge tube, add ice cold acetone to give a final 5 concentration of 90% (v/v) and mix well. Allow proteins to precipitate at-20oC for 2 h. The yield of the protein depends on the concentration of acetone.
Proteins were collected by centrifugation for 20min. at +5ºC and 5000g. The supernatant were discarded and precipitate washed once with ice cold acetone and centrifuge as above.
The resulting precipitates were carefully dried in a stream of N2 and add 400 μl of the lysis solution and mixed well.
The mixture of precipitates was kept in dialysis solution overnight against 25 ml of the modified lysis solution.
Centrifuge the dialysed sample at 8000g for 10 min. Add appropriate ampholytes to the clear supernatant to give a final 2% (v/v) concentration and store at –20ºC in suitable aliquotes. The suitability depended on the detection method to be used following the 2-D electrophoresis.

Extraction of chlorophyll (Arnon, 1949) [2]

One gram of finely cut fresh leaves were taken and ground with 20 – 40ml of 80% acetone. It was then centrifuged at 5000 –10000rpm for 5mins. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. The absorbance of the solution was red at 645 nm and 663 nm against the solvent (acetone) blank.

Estimation of chlorophyll content

The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:
Total Chlorophyll: 20.2(A645) + 8.02(A663)
Chlorophyll a: 12.7(A663) – 2.69(A645)
Chlorophyll b: 22.9(A645) – 4.68(A663)

Result

The present study was carried out to isolate and identify the bacterial species from collected root nodules and soil at STET Women's College Garden, Mannargudi, Thiruvarur (Dt), TamilNadu, India. The effect of different liquid bio fertilizers on the growth and productivity of *Vigna mungo* L. were studied. The results shown that viability of bacterium to decline during storage of bio fertilizer but did not significantly reduce the effect on growth and production of plant.

Isolation and identification of bacteria

Serial dilution technique was used to isolate the bacteria. Gram staining, Motility test and Biochemical tests, Indole,

MR-VP, Citrate Utilization test, Oxidase test, Catalase test, Triple Sugar Iron test and Carbohydrate Fermentation test were used to identify bacterial species. Gram negative, motile, rod shaped bacteria showing positive result for indole, MR, triple sugar iron test and catalase test and negative results for VP, citrate and Carbohydrate fermentation test. The organism was identified as *Rhizobium sp*. Gram negative, motile, rod shaped bacteria showing positive result for triple sugar iron test, Carbohydrate fermentation test and Catalase test and negative result for VP, Indole, MR and Citrate. The organism was identified as *Azospirillum sp*.Gram positive, motile, spherical shaped bacteria showing positive result for Indole, MR, Citrate and Catalase test and negative test for VP test. The organism identified as *Azotobacter*. Based on the results obtained above, the organisms were confirmed using Bergey's manual of Determinative Bacteriology.

Table 1

S. No	Characteristics	*Rhizobium sp*	*Azospirillum sp*	*Azotobacter sp*
1	Indole	+	-	+
2	MR	+	-	+
3	VP	-	-	-
4	Citrate	-	-	+
5	Catalase	+	+	+
6	TSI	+	+	+
7	Gram staining	-	-	-
8	Motility	Motile	Motile	Motile
9	Shape	Rod	Rod	Spherical

Preparation of liquid bio fertilizer

All isolated microorganisms were prepared, as liquid bio fertilizer. The bacterial species are prepared as liquid microbial consortium. Nutrient broth was used for the liquid consortium preparation. Now the liquid consortium was ready to use of liquid bio fertilizer.

Fig 1

Viability of bacteria

The results of viability test for bacteria during storage for 0, 1, 2 and 3 months were presented in Table-3. Initially viability of bacterium was high and then it was stable until two months of storage. After two months, the viability of bacteria present in liquid consortium was declined.

Table 2

Species	Storage period			
	0	1	2	3
Rhizobium sp	$1.9.10^{7}$	$2.3.10^{6}$	$2.5.10^{5}$	$1.8.10^{5}$
Azospirillum sp	1.5×10^{6}	1.25×10^{6}	3×10^{5}	1.9×10^{5}
Azotobacter sp	1.7×10^{6}	3×10^{6}	2×10^{6}	1.2×10^{5}

Field application

The efficiency of liquid bio fertilizers on growth *Vigna mungo* was stidied. The seed inoculation with liquid bacterial bio fertilizers was planed as, T1- *Rhizobium sp*, T2-*Azospirillum sp*, T3-*Azotobacter sp,* T4- *Rhizobium sp* + *Azospirillum sp* + *Azotobacter sp* and T5-Control. The height of the plant (in cm), number of leaves (per plant), number of flowers (per plant), root length (in cm), number of roots (per plant), number of seeds (in plants), number of root nodules (per plant), number of pods (per plant) and yield (seed and gram) was measured at 60 days, after sowing, among the overall treatments T4 was showed better response for combined inoculations than the other treatment and control.

Height of the plant (in cm)

At 30th day, maximum height of the plant was recorded in T4 (13±2.3) the combined inoculations, followed by other treatments, T1 (8±5.1), T2 (7±3.3), T3 (7±2.2) and T5 (6±2.2). (Plate-VII) and (Figure-1).On 45th day, maximum height of the plant was observed in combined inoculation T4 (16±2.4) followed by T1 (12±5.1), T2 (11±2.2), T3 (11±23) and T5 (8±2.7). (Plate-VII) and (Figure-1).On 60th day, maximum height of the plant was shown by T4 (25±2.9) followed by T1 (23±2.9)**,** T2 (21±3.9), T3 (21±34) and T5 (16±8.2).

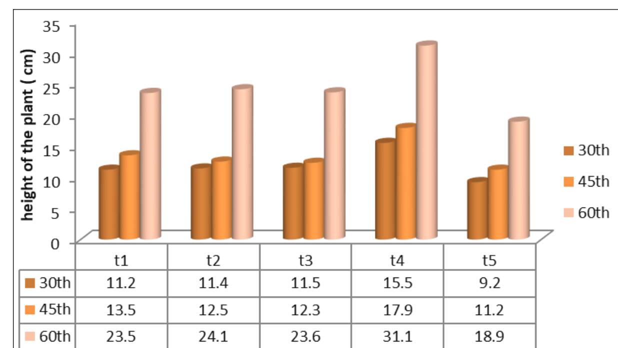

Fig 2

Number of leaves (per plant)

At 30th day, maximum number of leaves in the plant was recorded in T4 (9±4.5) the combined inoculation, followed by T1 (8±2.1), T2 (7±0.2), T3 (7±1.2) and T5 (5±2.3).On 45th day, number of leaves in the plant was observed in combined inoculation of T4 (12±2.3) followed by T1 (11±2.3), T2 (10±4.9), T3 (8±4.9) and T5 (7±3.5).On 60th day, number of leaves in the plant was shown by T4 (21±5.4) followed by T1 (19±2.8), T2 (17±2.6), T3 (18±9.6) and T5 (15±7.8).

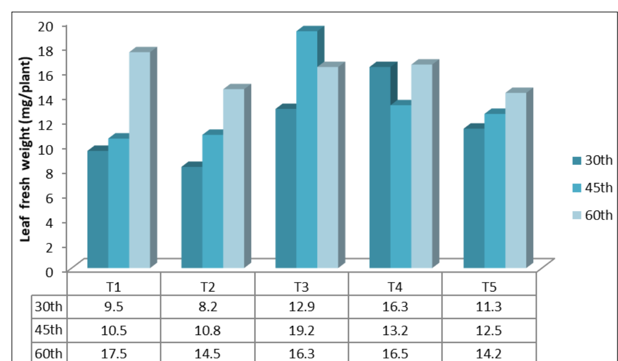

Fig 3

Number of flowers (Per plant)

On 45th day, maximum number of flowers in the plant was recorded in T4 (16±2.3) followed by other liquid bio fertilizer treatments T1 (12±4.5), T2 (11±6.4), T3 (9±9.8) and T5 (7±7.3).

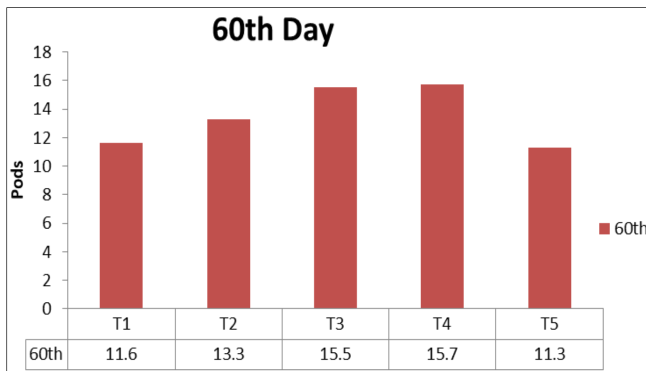

Fig 4

Number of root nodules (Per plant)

Among the overall treatments on 30th day, maximum number of root nodules were recorded combined inoculations such as, T4 (7±6.5) followed by T1 (6±4.5), T2 (5±2.6) T3 (5±1.2) and T5 (4±1.2). (Figure-7). Among the overall treatments on 45th day, maximum number of root nodules were recorded in combined inoculation such as T4 (8±6.7) followed by T1 (7±4.7), T2 (6±8.8) and T3 (5±1.5) and T5 (4±1.5). (Figure-7). At 60th day maximum number of root nodules were recorded in combined inoculation such as T4 (20±8.5) followed by T1 (19±4.5), T2 (18±4.2) and T3 (16±2.5) and T5 (15±2.5).

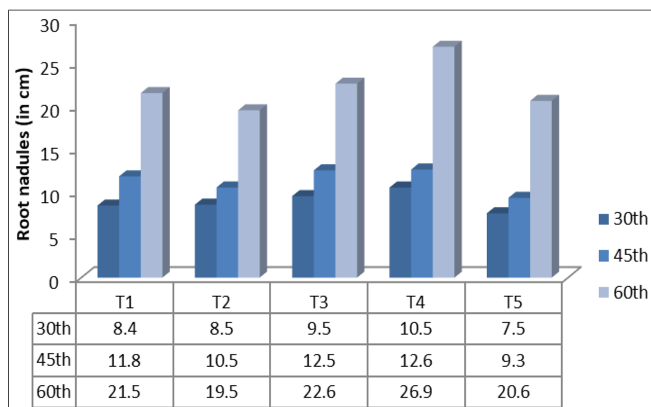

Fig 5

Root length (In cm)

On 30th day, maximum number of root length of the plant was observed in combined inoculation of T4 (8±1.2) followed by T1 (7±4.5), T2 (6±3.5) T3 (5±1.5) and T5 (4±3.5). (Plate-VI) and (Figure-6).At 45th day maximum number of root length of the plant was observed in combined inoculation of T4 (8±5.7) followed by T1 (6±4.2), T2 (5±0.8), T3 (5±4.3) and T5 (4±4.7). (Plate-VI) and (Figure-6).At 60th day maximum number of plant root length of the plant was observed in combined inoculation of T4 (12±4.5) followed by T1 (11±4.5), T2 (11±1.2), T4 (9±2.5) and T5 (7±2.1). (Plate-VI) and (Figure-6)

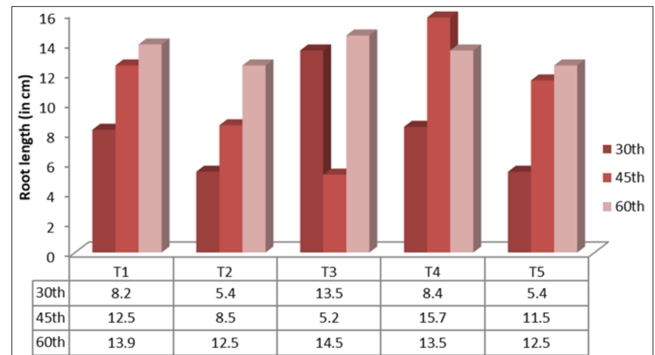

Fig 6

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) followed by T1 (6±9.5), T2 (4±1.5), T3 (4±9.8) and T5 (3±3.8). (Plate-V) and (Figure-5)**.**At 45th day, maximum number of shoot length in plant was observed in combined inoculation of T4 (8±1.2) followed by T1 (6±7.6), T2 (5±4.5), T3 (5±5.7) and T5 (4±7.6). (Plate-V) and (Figure-6)**.**At 60th day, maximum number of shoot length in plant was observed in combined inoculation of T4 (13±4.1) followed by T1 (11±6.7), T2 (9±4.2), T3 (10±1.5) and T5 (7±2.5). (Plate-V) and (Figure-6)

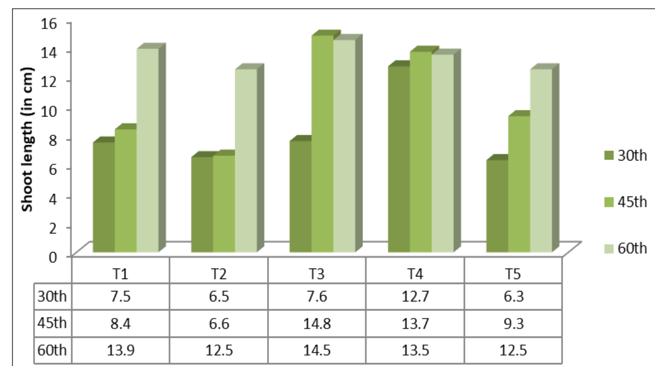

Fig 7

Number of seeds (Gm per plant)

On 60th day, maximum level seeds were observed in T4 (19±9.5) followed by other treatments T1 (17±4.6), T2 (16±0.2), T3 (14±0.9) and T5 (12±0.9).

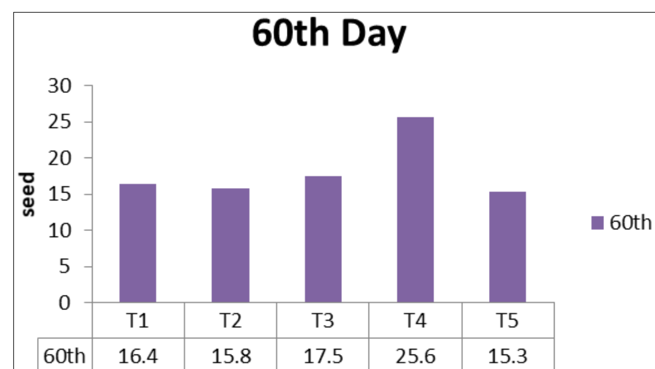

Fig 8

Leaf fresh weight (Mg per plant)

At 30th day, maximum level of leaf fresh weight was recorded in combined inoculations, T4 (9±8.7) and followed

by other treatment T1 (8±7.5), T2 (7±6.8), T3 (6±5.7) and T5 (5±2.4). (Figure-3).At 45th day, maximum level of leaf fresh weight was recorded in combined inoculations, T4 (12±1.2) and followed by other treatment T1 (11±3.9), T2 (10±1.2), T3 (8±9.8) and T5 (7±1.5). (Figure-3).At 60th day, maximum level of leaf fresh weight was recorded in combined inoculations, T4 (15±4.5) and followed by other treatment T1 (14±5.2), T2 (13±3.2), T3 (12±2.3) and T5 (11±1.5).

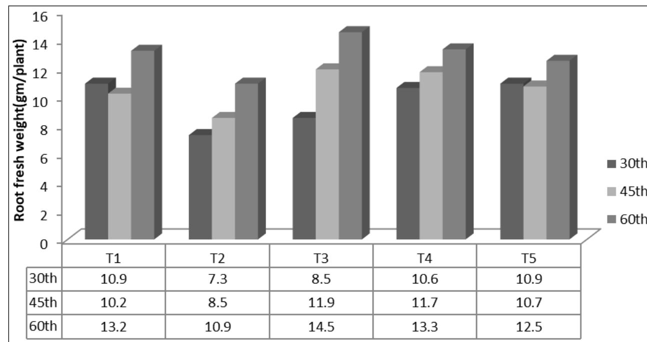

Fig 9

Leaf dry weight (Mg per plant)

At 30th day, maximum level of leaf dry weight was observed in combined inoculations of T4 (9±7.2) and followed by other treatment T1 (8±6.5), T2 (6±5.5), T3 (5±4.7) and T5 (3±2.8). (Figure-4).At 45th day, maximum level of leaf dry weight was observed in combined inoculations of T4 (9±5.6) and followed by other treatment T1 (7±5.6), T2 (6±7.2), T3 (6±2.2) and T5 (5±5.9). (Figure-4).At 60th day, maximum level of leaf dry weight was observed in combined inoculations of T4 (13±5.2) and followed by other treatment T1 (12±3.5), T2 (11±4.9), T3 (11±1.5) and T5 (9±4.2).

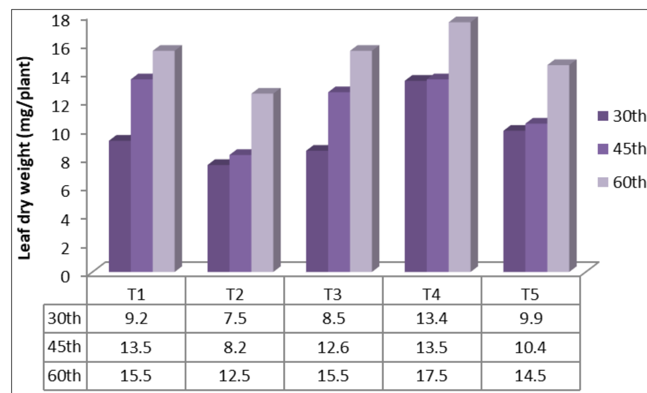

Fig 10

Number of pods (Per plant)

In 60th day maximum level pods were observed in T4 (14±2.3) the combined inoculation, followed by other treatments T1 (12±1.5), T2 (11±4.5), T3 (8±2.2) and T5 (6±3.5).

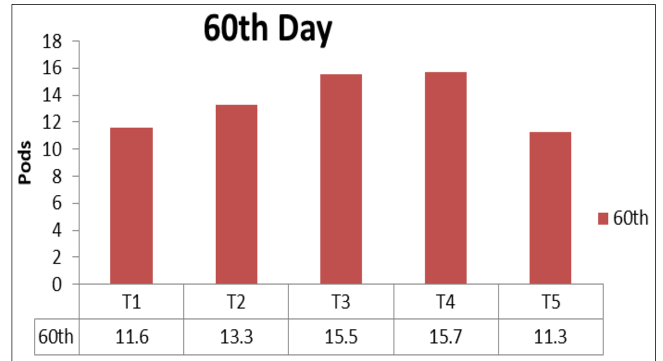

Fig 11

Yield (Seed in gram)

On 60th day, maximum level of yield was observed in combined inoculation of treatment such as T4 (19±9.5) followed by other treatments T1 (17±4.6), T2 (16±1.2), T3 (12±0.9) and T5 (9±3.2).

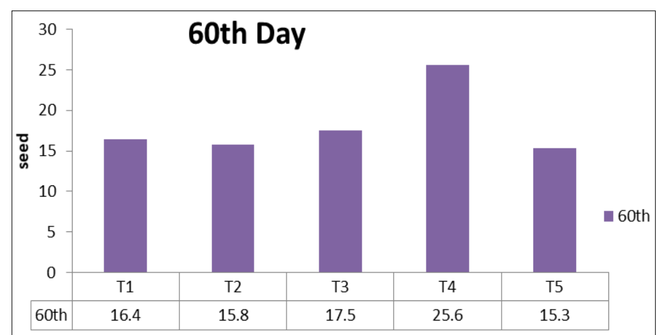

Fig 12

Carbohydrate estimation

The result showed the carbohydrate content in table-1. The maximum amount carbohydrate were found in T4 (consortium) and the minimum amount of carbohydrate content were found in T5 (control). The difference in the amount of carbohydrate was due to nitrogen application. The application of nitrogen is increase the carbohydrate content. If the less application of nitrogen is decrease the amount of carbohydrate content.

Chlorophyll

Result of the present study have been presented in table-1. In treated plant T4 (consortium) leaves have higher amound of chlorophyll then the other treatment (single inoculum) and control. Similarly, the lowest chlorophyll content was observed in T5 (control) untreated plant of *Vigna mungo.*

Protein estimation

Result of the present study have been presented in table-1. In treated plant T4 (consortium) have higher amound of protein content then the other treatment (single inoculum) and control. Similarly, the lowest protein content was observed in T5 (control) untreated plant of *Vigna mungo.*

Table 3

Treatment	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Carbohydrates	Protein
T1	0.453±0.43	0.0389±0.86	0.0452 ±012	4.45±0.49	0.389± 0.86
T2	0.497±0.034	0.0423±0.56	0.0490±0.089	4.69± 0.034	3.367±0.040
T3	0.510±0.030	0.0484±0.049	0.0521±0.423	5.45± 0.030	3.421±0.087
T4	0.643± 0.56	0.0598±0.067	0.0678±0.087	6.643± 0.56	4.620±0.057
Control	0.0321±0.09	0.0311±0.076	0.412± 0.56	0.0321± 0.09	4.265±0.051

Finally, our results showed that the combined inoculation of bacterial (*Rhizobium sp + Azospirillum sp+ Azotobacter sp*) liquid bio fertilizer has given a good response when compared to other treatments which were followed by *Rhizobium sp*, *Azospirillum sp, Azotobacter sp* and Control. Because the liquid microbial consortium consisted of nitrogen fixing microbes, plant growth hormones producing microbes, heavy metal mobilization microbes. Rhizobia are associated with rhizosphere, the part of soil under the influence of plant roots and their exudates. *Azospirillum sp* fix nitrogen in the rhizospheric soil. It has the ability to induce abuntant roots in several plants and it has ability the in mineral uptake of signal molecules. So, the bacteria give good response for growth and yield of crop. However applications are based on their ability to supply and mobilize plant nutrients, control plant diseases and promote plant growth and development. The bacteria were present in soil. Liquid bio fertilizers preparation was easy and low cost one.

References

1. Aamir Hamid Khan, Nasir Mehmood Minhas, Muhammad Javaid Asad, Azhar Iqbal, Muhammad Ilyas, Raja Tahir Mahmood. Estimation of Protein, Carbohydrate, Starch and Oil Contents of Indigenous Maize (*Zea mays* L.) Germplasm. j. of European academic research, 2014, 2.
2. Arnon DI. Copper enzymes in isolated chloroplasts poly phenol oxidase in beta vulgaris. Plant Physiol,1949:24:1-15.
3. Chandra K, Greep S, Ravindranath P, Srivathsa RSH. Liquid Biofertilizers, Ministry of agriculture department of agriculture & co-operation, Government of India, 2010.
4. Croft H. Leaf chlorophyll content as a proxy for leaf photosynthetic Capacity, Global Change Biology,2017:23(9):3513-24.
5. Gupta SP. Measures of central value and measures of dispersion. In: statistical methods, 2004, 180-181.
6. Gupta SP, Measures of central value and measures of dispersion. In statistical methods, 2004, 180-181.
7. Hegde SV. Liquid bio-fertilizers in Indian agriculture. Bio-fertilizer newsletter, 2008, 17-22.
8. Mahdi SS, Hassan GI, Samoon SA, Rather HA, Dar SA, Biofertilizers in organic agriculture, J. phytol,2010:2:42-54.
9. Mia, Shamsuddin ZH, *Rhizobium* as a crop enhancer and biofertilizer for increased cereal production, African Journal of Biotechnology,2010:9:6001-6009.
10. Mishra DJ, Singh Rajvir. Role of biofertilizer in organic agriculture, Res J. of recent sciences,2013:2:39-41.
11. Norris JR, Ribbons DW. Methods in microbiology, Academic Press New York,1972:4:795-799.
12. Pindi PK, Satyanarayana SD. Liquid microbial consortium-a potential tool for sustainable soil health. Journal of Biofertil Biopesticides,2010:3:4-6.
13. Senthilkumar PK, Sivagurunathan P. Comparative effect on bacterial biofertilizers on growth yield of green gram (*Phaseolus radiate L.*) and cow pea (*Vigna siensi Edhl.*), International journal of current microbiology and applied sciences,2012:1:34-39.
14. Somasegaran P, Hoben HJ. Handbook for Rhizobia. Methods in Legume- Rhizobium Teachnology. Heidelberg, NY: Springer. 10.1007/978-1-4613-8375-8, 1994.
15. Subba-Rao NS. Phosphate Solubilization by Soil Microorganisms. In: Advances in Agricultural Microbiology (Ed: Subba-Rao, N.S.). Oxford and IBH, New Delhi, 1986, 295-303.
16. Vessey JK. Plant growth promoting *Rhizobacteria* as biofertilizers. Plant Soil,2003:255:571-586.
17. Waxman SA. Dilution plate technique. J. bacteriol,1920:1:139-140.