# Beejamrutha: The Agricultural Bioenhancer

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*Abstract*— Agriculture is most important district of Indian economy. Globally India ranks second in agriculture. Now a days, India has made much progress in agriculture field. Agriculture is primary source of livelihood for about 58% of Indian population. India is the most populated country, so there is an increased demand of food as well as land. There are two types of supplements for agriculture, specifically fertilizer and pesticides. Organic agriculture is a way of farming with an aim of conserving the natural resources through the natural agronomic practices and the use of locally available low cost inputs like cow dung, urine etc, in order to maintain soil fertility and conserve the biodiversity. Organic production is the safest way of agricultural practice. Hence the eco-friendly safe modern technologies which are acceptable to the farmers. 'Beejamrutha' is a bio fertilizer traditionally prepared by mixing cow products, water, soil, and lime. It is a natural product helpful for the plant growth. The beneficial microorganisms present in 'Beejamrutha' protect the plants from harmful soil and seed borne pathogens and gives immunity to the plants during monsoon. The main purpose of 'Beejamrutha' is to remove the root fungus. The microorganisms present in 'Beejamrutha' enhances the nitrogen fixing, phosphate solubilizing capacity of plants. The bacteria were isolated from 'Beejamrutha' and tests for their beneficial traits. These isolates perform nitrogen fixation, phosphate solubilization and IAA (Iodole Acetic Acid) production. The beneficial microorganisms, fungi, actinomycetes, nitrogen fixers, phosphate solublizers and IAA enhancing bacteria from 'Beejamrutha' were isolated and characterized and the influence of 'Beejamrutha' on plant height of wheat ( Triticum aestivum) were studied. It does not having any health hazard effect to human being. So the use of 'Beejamrutha' is very safe and it is harmless.

***Keywords:*** Beejamrutha, nitrogen fixing bacteria, phosphate solubilizing bacteria, IAA enhancing bacteria

## I. INTRODUCTION

*A. Applications:*

1) The isolated species from 'Beejamrutha' are tested for nitrogen fixation, phosphate solubilization and IAA enhancers.
2) 'Beejamrutha' fix atmospheric nitrogen in the soil and make available to the plants.
3) They solubilise the insoluble forms of phosphates like tricalcium iron and aluminium phosphate into available form.
4) They produce hormones like IAA and GA which promote root and shoot development.
5) They remove root fungus, soil and seed borne diseases.
6) 'Beejamrutha' gives immunity to the plants during monsoon.
7) It increases the nutrient availability and improves the crop yield.

## II. AIM AND OBJECTIVES OF THE WORK

*A. Aim:*

"Analysis of 'Beejamrutha' as a agricultural bioenhancer."

*B. Objectives:*

1) To Prepare 'Beejamrutha' and isolate the bacteria from 'Beejamrutha'.
2) To study the morphological Characters, Gram nature, and Mobility of isolated bacteria.
3) Isolation of Fungi and Actinomycetes from 'Beejamrutha'.
4) Isolation of nitrogen fixing, Phosphate solubilizing, IAA enhancing bacteria from 'Beejamrutha'.
5) To study the effect of 'Beejamrutha' on plant height on wheat (Triticum aestivum).

## III. REVIEW OF LITERATURE

1) www.palekarzerobudgetspiritualfarming.org.

The preparation of 'Beejamrutha' is taken from ZBNF.

2) Beejamrutha: A source of beneficial bacteria.

M.N. SREENIVASA, NAGARAJ NAIK AND S.N. BHAT
Institute of Organic Farming, University of Agricultural Sciences, Dharwad.

The serial dilution and standard plate count method used for total bacteria, fungi, actinomycetes and other biochemical groups viz. free living nitrogen fixers and phosphate solubilizers using nutrient agar, martin's rose bengal agar, kuster's agar, norm's nitrogen free media and pikovskaya's media respectively. The plates were incubated at 28ºC for one week.

3) Functional characterization of microbial isolates of Beejamrutha and their influence on plant growth and yield of chilli (Capsicum annuum L.)

M. N. SREENIVASA, N.S HEBSUR, V.K. DESHPANDE, P. JONES NIRMALNATH
Thesis submitted to the University of Agricultural Sciences, Dharwad.

a) Palekar (2006) reported that Beejamrutha is not only a source of nutrients, but it is a product which contains ingredients viz. cow farmers for seed treatment which was found to increase seed germination and growth as it contains growth hormones and beneficial microflora.
b) Sreenivasa etal. (2010) reported that the beneficial microorganisms present in Beejamrutha produced IAA and GA resulted in improvement in seed germination and seedling length and seed vigour in soybean.

## IV. MATERIAL

The 'Beejamrutha' were prepared by using desi cow urine, dung, soil water and lime, nutrient agar, czapek dox agar medium, yeast glucose agar medium, nitrogen free mannitol agar medium, katznesion and bose (kb) medium, Std. IAA, salkowaski reagent.

 

Fig. 1: Prepared Beejamrutha

## V. METHODS

### *A. Isolation of bacteria from 'Beejamrutha':*

For isolation of bacteria serial dilution method is used. In this method take 10 test tubes mark it as $10^{-1}$, $10^{-2}$, $10^{-3}$....,$10^{-10}$ and add 10 ml sterile distilled water in first tube and 9 ml distilled water in remaining 9 tubes. After this 1 ml of 'Beejamrutha' is transfer to the 2nd tube. The procedure was repeated up to the last tube. After dilution take 0.1 ml of dilution of each tube and spread on Nutrient Agar Medium plate. The plates were incubated in an inverted position at 28°c for one week. After incubation 5 well isolated colonies A, B, C, D, E were selected and morphological characteristics, gram nature and mobility were studied. Colonies grown on respective plates were sub cultured on respective agar slants and preserve it in freezer for further studies of these organisms. These isolates were checked for Nitrogen Fixation, Phosphate Solubilization and IAA enhancing bacteria.

### *B. Isolation of Fungi and Actinomycetes from 'Beejamrutha':*

For isolation of both Fungi and Actinomycetes serial dilution and spread plate technique was used. For isolation of Fungi Czapek Dox Medium was used and for isolation of Actinomycetes Yeast Glucose Agar Medium was used. The respective plates were incubated at 28°C for 4 days. After incubation we get Fungi and Actinomycetes colonies.

### *C. Isolation of Nitrogen fixing bacteria:*

Nitrogen fixing bacteria plays important role in environment. As they fix the atmospheric nitrogen into soluble form of nitrogen that is ammonia, which is utilized by plants easily. It increases the crop yield. For isolation of nitrogen fixing bacteria, Nitrogen Free Mannitol Agar medium was used. Serial dilution and spread plate technique was used for the isolation purpose. Respective dilutions of isolated bacteria A, B, C, D, E were spread on the medium and plates were incubated at 28±2°C for 4 days. After incubation the plates were observed for nitrogen fixing bacteria. The bacteria 'E' having the capacity of nitrogen fixation.

### *D. Isolation of Phosphate Solubilizing bacteria:*

PSB (Phosphate Solubilizing Bacteria) having capacity to convert insoluble phosphorus into soluble form. PSB can improve the quality of soil and also improves plant growth. For isolation of phosphate solubilizing bacteria, Katznesion and Bose (KB) medium was used. Serial dilution and spread plate technique were used for the isolation purpose. Respective dilutions of isolated bacteria A, B, C, D, E were spread on the medium and plates were incubated at 28±2°C for 4 days. After incubation the plates were observed for phosphate solubilizing bacteria. The bacteria 'B' and 'D' having the capacity of phosphate solubilization.

### *E. Quantitative assay of IAA (Indole Acetic Acid):*

IAA (Indole Acetic Acid) is an important growth hormone which stimulates root development, stimulates cell growth and cell expansion. When IAA reacts with Salkowaski reagent in presence of orthophosphoric acid a pink colored complex was formed. The intensity of pink color is directly proportional to the concentration of IAA present and O.D was measured at 530 nm. The five isolates of bacteria 'A', 'B', 'C', 'D', 'E' were tested for IAA production. For assay take six clean dry test tubes and add 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of std IAA serially. Adjust the volume to 1 ml of first five tubes by adding distilled water. In seventh tube take 1 ml sample (enriched solution of bacteria 'A') without distilled water. In all the seventh tubes add two drops of orthophosphoric acid. Then add 4ml of Salkowaski reagent in all seven tubes. Keep the set in dark for 30 min for formation of pink color and O.D is measured at 530 nm. Plot the graph of O.D vs. conc. and take intercept on X-axis by considering O.D of sample on Y-axis to find out the conc. of IAA in sample. Repeat the procedure for remaining enriched isolates B, C, D and E. The bacteria 'A' and 'D' shows the positive test for IAA production.

### *F. Influence of 'Beejamrutha' on plant height on Wheat (Triticum aestivum):*

The influence of 'Beejamrutha' on plant height of wheat (Triticum aestivum). For this, the seeds of wheat are soaked in beejamrutha for about 48 hrs and named as 'with beejamrutha' and non-treated seeds named as 'without beejamrutha'. The two trays are compared after 10 days of interval. the plants 'with beejamrutha' shows relatively more as compared to 'without beejamrutha' and also resist to root fungus. From this we can conclude that beejamrutha contains growth promoting substances as well as it resist to root fungus.

## VI. RESULT AND DISCUSSION

For isolation of bacteria from 'Beejamrutha' serial dilution and spread plate technique were used. on a Nutrient Agar Medium well isolated colonies were observed. The isolated five specimen namely A, B, C, D and E were selected for the further studies. The further studies carried morphological characters, gram nature, mobility, nitrogen fixation, phosphate solubilization, IAA production of isolated species were studied. As well as beneficial fungi and actinomycetes also isolated from 'Beejamrutha' which is as follows:

 

*A. Isolated species:*

Fig. 2: Isolated bacterial species from 'Beejamrutha

Morphological characters, gram nature and mobility of selected species:

*1) Specimen 'A'*

| Size | Shape | Color | Margin |
|---|---|---|---|
| 4mm | Circular | Pale | Entire |
| Elevation | Opacity | Surface | Consistancy |
| Convex | Opaque | Smooth | Moist |

| Gram nature | Motility |
|---|---|
| Gram negative [cocci] | Non motile |

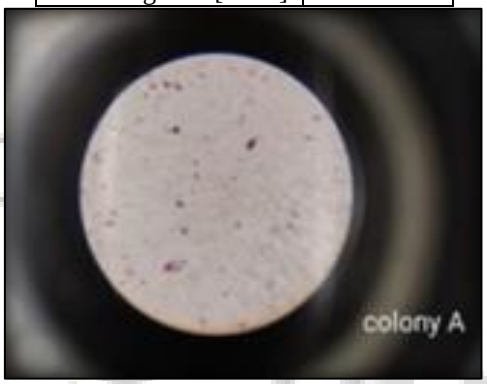


Fig. 3: Specimen 'A'

| Size | Shape | Color | Margin |
|---|---|---|---|
| 3mm | Circular | Pale yellow | Entire |
| Elevation | Opacity | Surface | Consistancy |
| Flat | Opaque | Smooth | Moist |

| Gram nature | Motility |
|---|---|
| Gram negative [cocci] | Non motile |

*2) Specimen 'B'*

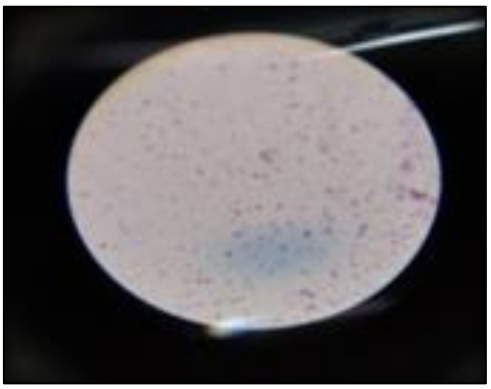

Fig. 4: Specimen 'B'

*3) Specimen 'C'*

| Size | Shape | Color | Margin |
|---|---|---|---|
| 5mm | Circular | Pale | Entire |
| Elevation | Opacity | Surface | Consistancy |
| Flat | Opaque | Smooth | Moist |

| Gram nature | Motility |
|---|---|
| Gram negative [cocci] | Non motile |

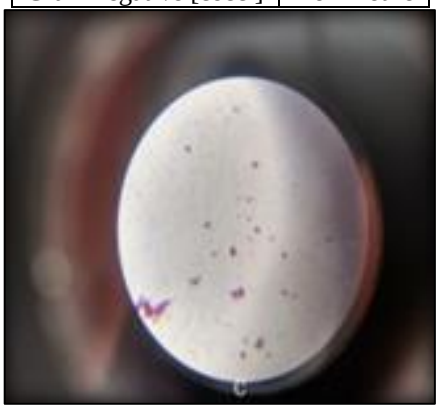

Fig. 5: Specimen 'C'

*4) Specimen 'D'*

| Size | Shape | Color | Margin |
|---|---|---|---|
| 2mm | Circular | Pale yellow | Entire |
| Elevation | Opacity | Surface | Consistancy |
| Flat | Opaque | Smooth | Moist |

| Gram nature | Motility |
|---|---|
| Gram negative [cocci] | Non motile |

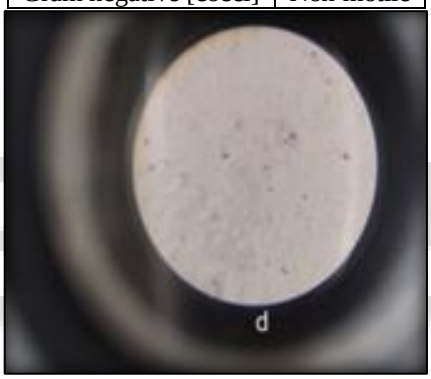


Fig. 6: Specimen 'D'

*5) Specimen 'E'*

| Size | Shape | Color | Margin |
|---|---|---|---|
| 3mm | Irregular | Pale yellow | Irregular |
| Elevation | Opacity | Surface | Consistancy |
| Flat | Opaque | Rough | Moist |

| Gram nature | Motility |
|---|---|
| Gram negative [cocci] | Non motile |


Fig. 7: Specimen 'E'

*B. Isolated Fungi and Actinomycetes from 'Beejamrutha':*

For isolation of Fungi Czapek Dox Medium was used and for isolation of Actinomycetes Yeast Glucose Agar Medium

 

was used. Serial dilution and spread plate technique were used for isolation purpose.

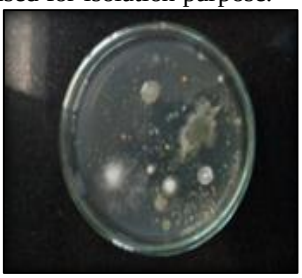

Fig. 8: Isolated Fungi

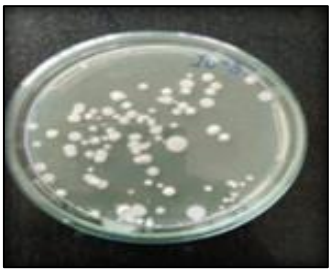

Fig. 9: Isolated Actinomycetes

*C. Isolated specimen 'E' showing nitrogen fixing capacity:*

For isolation of nitrogen fixing bacteria from 'Beejamrutha', Nitrogen Free Mannitol Agar medium is used.

Fig. 10: Isolated nitrogen fixing specimen 'E'

*D. Isolated species 'B' and 'D' showing phosphate solubilizing capacity:*

For isolation of phosphate solubilizing bacteria, Katznesion and Bose (KB) medium was used. Serial dilution and spread plate technique were used for the isolation purpose.

Fig. 11: Phosphate solubilizing specimen 'B'

Fig. 12: Phosphate solubilizing specimen 'D'

*E. Quantitative Assay of IAA (Indole Acetic Acid):*

The five isolated bacteria A, B, C, D and E were tested for IAA production.

*1) Quantitative analysis of IAA : Specimen 'A'*

Std. conc. of IAA : 500 µg/ml

| Sr. No. | Std IAA ( ml) | D/w (ml) | Orthopho-Sphor-ic acid | Salkowas-ki Reagent ( ml ) | | O.D at 530 nm. |
|---|---|---|---|---|---|---|
| 1 | 0.0 | 1.0 | Add 2 drops Of Orthoph-osphoric acid in all tubes | 4 | Keep all the tubes in dark for about 30 min for development of pink color. | 0.00 |
| 2 | 0.2 | 0.8 | | 4 | | 0.16 |
| 3 | 0.4 | 0.6 | | 4 | | 0.30 |
| 4 | 0.6 | 0.4 | | 4 | | 0.40 |
| 5 | 0.8 | 0.2 | | 4 | | 0.52 |
| 6 | 1.0 | 0.0 | | 4 | | 0.56 |
| Samp-le | - | - | | 4 | | 0.22 |

Fig. 13: Positive test for IAA production of specimen 'A'

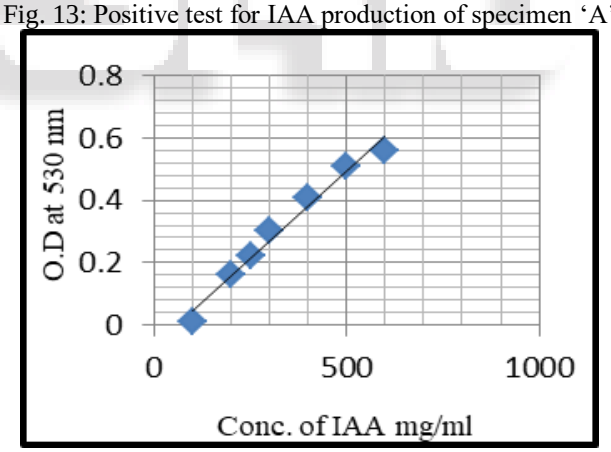


Fig. 14: The IAA conc. in the sample is 250 µg/ml.

*2) Quantitative analysis of IAA : Specimen 'D'*

Std. conc. of IAA : 500 µg/ml

| Sr. No. | Std IAA ( ml ) | D/w (ml) | Ortho pho-Sphor-ic acid | Salkowas-ki Reagent ( ml ) | | O.D at 530 nm. |
|---|---|---|---|---|---|---|
| 1 | 0.0 | 1.0 | Add 2 drops Of Ortho ph-ospho | 4 | Keep all the tubes in dark for about 30 min for develop | 0.00 |
| 2 | 0.2 | 0.8 | | 4 | | 0.13 |
| 3 | 0.4 | 0.6 | | 4 | | 0.19 |
| 4 | 0.6 | 0.4 | | 4 | | 0.30 |
| 5 | 0.8 | 0.2 | | 4 | | 0.43 |
| 6 | 1.0 | 0.0 | | 4 | | 0.50 |
| Sampl | - | - | | 4 | | 0.26 |

| | | | | | | |
|---|---|---|---|---|---|---|
| e | | | ric acid in all tubes | | ment of pink color. | |

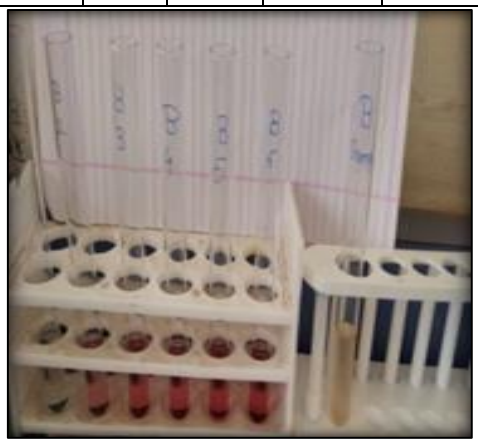

Fig. 15: Positive test for IAA production of specimen 'D'

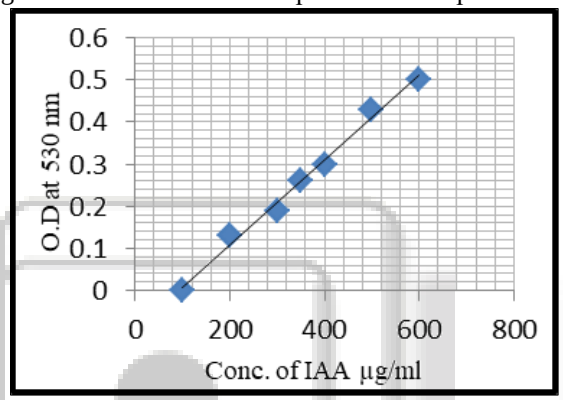


Fig. 16: The IAA conc. in the sample is 350 µg/ml.

*F. Infiuence of 'Beejamrutha on plant height on Wheat (Triticum aestivum) :*

The seeds of wheat are soaked in beejamrutha for about 48 hrs and named as 'with beejamrutha' and non treated seeds named as 'without beejamrutha'. The two trays are compared after 10 days of interval. the plants 'with beejamrutha' shows relatively more as compared to 'without beejamrutha'.

Fig. 17: Non-treated seeds of Wheat

Fig. 18: Treated seeds of Wheat


Fig. 19: Effect of 'Beejamrutha' on plant height of Wheat

*G. Effect of 'Beejamrutha' on plant height on Wheat:*

| Sr no. | Height of plants with 'Beejamrutha' (cm) | Height of plants without 'Beejamrutha' (cm) |
|---|---|---|
| 1. | 38.5 | 29.1 |
| 2. | 32 | 28 |
| 3. | 33 | 24.7 |
| 4. | 31 | 15.5 |
| 5. | 32 | 26 |
| 6. | 39 | 28 |
| 7. | 33 | 29.7 |
| 8. | 30.6 | 28.5 |
| 9. | 27.3 | 30.3 |
| 10. | 30.2 | 11.2 |

*H. Beneficial activities of isolated specimens:*

| Bacteria | Nitrogen fixing capacity | Phosphate solubilizing capacity | IAA producing capacity |
|---|---|---|---|
| A | - | - | + |
| B | - | + | - |
| C | - | - | - |
| D | - | + | + |
| E | + | - | - |

## VII. Conclusion

1) 'Beejamrutha' were found to have higher number of beneficial microorganisms. It contains beneficial fungi, actinomycetes.
2) The five isolates of bacteria 'A', 'B' ,'C', 'D', 'E' are tested for nitrogen fixers, phosphate solubilizers and IAA (Indole Acetic Acid) producers.
3) Bacteria 'E' having nitrogen fixing capacity.
4) Bacteria 'B' and 'D' having phosphate solubilizing capacity.
5) Bacteria 'A' and 'D' having IAA producing capacity. The bacteria 'A' having 250 µg/ml IAA production and bacteria 'D' having 350 µg/ml IAA production.
6) Significant variation in the plant height are seen as beejamrutha contains the IAA growth hormone which is involved in the root and shoot development.
7) Presence of such beneficial microorganisms and nutrient status have resulted in enhanced plant height in wheat (Triticum aestivum) indicating 'beejamrutha' as an efficient plant growth stimulant.

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