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Beejamrutha: A source for beneficial bacteria

M. N. SREENIVASA, NAGARAJ NAIK AND S. N. BHAT

Institute of Organic Farming,
University of Agricultural Sciences, Dharwad-58005, Karnataka, India.
E-mail: sreenivasa_mn@rediffmail.com

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Abstract : Use of Beejamrutha, a mix of cow dung, cow urine, water, lime and a handful of soil has been given importance in sustainable agriculture since age old days. It is also one such organic product helpful for the plant growth. The beneficial microorganisms present in beejamrutha are known to protect the crop from harmful soil-borne and seed-borne pathogens. Bacteria were isolated from beejamrutha and tested for their beneficial traits. These isolates were capable of N_2-fixation, P-solubilization and IAA, GA production in addition to suppression of *Sclerotium*. Among the free living N_2-fixers, isolate A_2B_2 registered highest amount of N_2 fixation (13.71 mg/g carbon source utilized) where as BPS3 released maximum amount of Pi (8.15 per cent) among phosphate solubilizing bacteria isolated from beejamrutha. The isolate BJ5 was found to produce highest amount of IAA (11.36 µg/25ml) and GA (3.13µg/25ml). Inoculation of the bacterial isolates from beejamrutha also resulted in improvement in seed germination, seedling length and seed vigour in soybean. Among the treatments, seeds inoculated with BJ5 has registered significantly higher seedling length and seedling vigour index while the seedling length and seedling vigour index was markedly lowest in control.

Key words: beejamrutha, indole acetic acid, gibberlic acid, seed vigour index, soybean

Introduction

India has been endowed with rich biodiversity, varied types of soil, copious rainfall and abundant sunshine. It is very much essential to develop a strong workable and compatible package of nutrient management through organic sources for various crops based on scientific facts, local conditions and economic viability (Kannaiyan, 2000). Among indigenous technologies used by farmers, use of beejamrutha has been given importance since age old days. Beejamrutha, a mix of cow dung, cow urine, water, lime and a handful of soil, a totally organic product helpful for the p`lant growth and protects the crop from harmful soil-borne and seed-borne pathogens. Smearing the seeds with beejamrutha before sowing control many diseases that attack the plant right from its seedling stage. At times, saplings are dipped in the beejamrutha before being transplanted. Presence of naturally occurring beneficial microorganisms predominantly bacteria, yeast, actinomycetes, photosynthetic bacteria and certain fungi were detected in cowdung (Swaminathan, 2005) which is one component of beejamrutha.

Material and methods

The present study was carried out at the Institute of Organic Farming, University of Agricultural Sciences, Dharwad, during the 2008-09. The materials used and the methods followed in carrying out the experiments are presented here.

Beejamrutha was prepared using the ingredients viz cow dung, cow urine, water and lime. Cowdung (5 kg) tied in a cloth was dipped in a bucket containing 50 liters of water overnight. Next day morning, the tied dung is frequently squeezed and dipped in the water. Five litres of cow urine, a handful of soil and 50g of calcium chloride was added to this extract.

The serial dilution and standard plate count method was used for isolation of total bacteria, fungi, actinomycetes and other biochemical groups viz free living N_2 fixers and P-solubilizers using nutrient agar, Martin's rose bengal agar, Kuster's agar, Norri's N free media and Pikovskaya's media respectively. The plates were incubated at 28 ± 2^0C for one week and the colony counts were recorded. The predominant bacterial colonies grown on respective plates were subcultured on nutrient agar slants for further use.

The major nutrients such as nitrogen, phosphorus and potassium present in beejamrutha were estimated by following microkjeldhal, vanadomolybdate and flame photometric methods respectively. Micronutrients present in beejamrutha were estimated using Atomic Absorption Spectrophotometer (AAS).

The capacity of nitrogen fixed in the broth culture by free living N_2 fixing isolates in beejamrutha was estimated by the microkjeldhal method described by Jackson (1973). The amount of Pi released in the broth was estimated at 10 days of incubation. The broth cultures were centrifuged at 10000 rpm for 10 minutes in a micro centrifuge to separate the supernatant from the cell growth and insoluble phosphate. The available P content in the supernatant was estimated by phosphomolybdic blue colour method.

All the seven isolates grown on nutrient agar were subjected to qualitative analysis for the production of Indole acetic acid (IAA) and Gibberlic acid (GA). Luria agar supplemented with 0.06 per cent sodium dodecyl sulphate and one per cent glycerol was prepared and plated. The overnight culture of each isolate spotted on those plates. The spotted plates were overlaid immediately with sterile disc of Whatman No.1filter paper. After 4 days of incubation period, the filter paper discs were removed from the plates and treated with

Salkowaski reagent. Bacteria producing IAA were identified by the formation of characteristic red halo around the colony on filer paper. The paper discs after treatment with salkowaski's reagent were viewed under UV light. The spots giving typical green fluorescence were taken as positive for GA production. Those isolates found positive for IAA and GA production were further used for the quantitative estimation of IAA and GA following the methods of Gorden and Paleg (1957) and Paleg (1965), respectively.

Further these isolates were tested for their biocontrol potential. The biocontrol test was done against the pathogen *Sclerotium* collected from the Department of Plant Pathology, UAS Dharwad. The pathogen was spotted in the center marked area in the petriplate containing media and test isolate was spotted on the side of pathogen and the plate was incubated for 3-4 days. The zone of inhibition indicates the ability of test isolate to suppress the growth of pathogen. Based on the zone of inhibition per cent inhibition was calculated.

The effect of bacterial cultures isolated from beejamrutha on seed germination and seedling vigour index in soybean was studied. For germination test, soybean seeds were dipped in broth of bacterial isolates for ten minutes and kept on germination paper. On 8th day, the germinated seeds were counted and their percent germination calculated.

Seedling length (cm): On eighth day of germination test, ten normal seedlings were taken out carefully at random from each treatments and measured from the tip of primary root to the tip of apical shoot. The average was calculated and expressed as mean seedling length in centimeters.

Seedling vigour index: The seedling vigour index was calculated adopting the method suggested by Abdul-Baki and Anderson (1973) and expressed in whole number treatmentwise.

Vigour index = Germination percentage x Seedling length

Results and discussion

The nutrient status of beejamrutha was estimated and given in table 1a. The population (cfu/ml) of total bacteria, fungi, actinomycetes, free living N_2-fixers and phosphate solubilising microorganisms in beejamrutha was 15.4 $\times 10^5$, 10.5 $\times 10^3$, 6.8 $\times 10^3$, 3.1 $\times 10^2$ and 2.7 $\times 10^2$ respectively (Table1b). Seven predominant colonies from Norri's N free media as free living N_2-fixers, seven predominant bacterial colonies from Pikovskaya's media as phosphate solubilising bacteria and seven general bacterial isolates from nutrient agar media were subcultured for further studies. The isolates grown on Norri's N free media and Pikovskaya's media were tested for the quantum of N_2 fixation and per cent Pi release respectively. Among the free living N_2-fixers, isolate A_zB_2 registered highest amount of N_2 fixation (13.71 mg/g carbon source utilized) where as BPS3 released maximum amount of Pi (8.15 per cent) among phosphate solubilizing bacteria isolated from beejamrutha (Table 2).

Seven major representative isolates from beejamrutha isolated on nutrient agar were purified and subcultured on the slants for further experiments. These isolates were examined for production of indole acetic acid (IAA) and gibberellic acid (GA) by qualitative and quantitative methods and the results are presented in table 3. The quantity of IAA produced by different strains ranged from 4.11 µg to 11.36 µg/25ml broth, where as the amount of GA produced ranged from 1.06 µg to 3.13 µg/25ml broth. Out of seven isolates, six isolates were capable of producing IAA and five isolates were capable of producing GA where as 4 isolates produced both IAA and GA. The isolate BJ5

Table 1a. Nutrient status of beejamrutha

Sl.No.	Parameter	Content
1.	pH	8.2
2.	EC (Soluble salt)	5.5 dSm^{-1}
3.	Total Nitrogen	40 ppm
4.	Total Phosphorus	155.3 ppm
5.	Total Potassium	252.0 ppm
6.	Total Zinc	2.96 ppm
7.	Total Copper	0.52 ppm
8.	Total Iron	15.35 ppm
9.	Total Manganese	3.32 ppm

Table 1b. Microbial load in beejamrutha

Sl. No.	Organisms	Colony count (cfu/ml)
1.	Bacteria	15.4 X 10^5
2.	Fungi	10.5 X 10^3
3.	Actinomycetes	6.8 X 10^3
4.	Phosphate solubising organisms	2.7 X 10^2
5.	N_2-fixers	3.1 X 10^2

Table 2. N_2-fixing and phosphate solubilisation capacity of bacterial isolates of beejamruth

Sl. No.	N_2-fixing isolates	Amount of N_2 fixed (mg/g carbon)	Phosphate Solubilising solates	Pi-released (%)
1.	AzB1	8.12	BPS1	1.37
2.	AzB2	13.71	BPS2	6.71
3.	AzB3	7.32	BPS3	8.15
4.	AzB4	10.88	BPS4	2.35
5.	AzB5	8.89	BPS5	7.25
6.	AzB6	12.36	BPS6	3.13
7.	AzB7	11.15	BPS7	3.12

Table 3. Indole acetic acid, gibberllic acid production and biocontrol potential of general bacteria isolated from beejamrutha

Sl. No.	Bacterial Isolates	IAA (µg/25ml)	GA (µg/25ml)	Biocontrol effect Result	Inhibition(%)
1	BJ1	6.12	1.06	+ve	75
2	BJ2	8.45	1.27	+ve	66
3	BJ3	-ve	2.23	-ve	Nil
4	BJ4	4.13	-ve	-ve	Nil
5	BJ5	11.36	3.13	+ve	88
6	BJ6	7.23	-ve	-ve	Nil
7	BJ7	10.04	3.01	+ve	81

was found to produce highest amount of IAA (11.36 µg/25ml) followed by BJ7 (10.04 µg/25ml) and the least quantity was produced by BJ4. In case of GA production also, BJ5 registered highest quantity (3.13 µg/25ml) followed by BJ7 (3.01 µg/25ml) and the lowest by BJ1. The biocontrol potential of selected isolates on *Sclerotium*, was conducted and the results are presented in t able 3. Among the seven isolates, four isolates (BJ1, BJ2, BJ5 and BJ7) showed positive effect in suppressing the growth of the pathogen. Maximum per cent inhibition (88) of *Sclerotium* was observed in BJ5 (Table 3). The results are in accordance with earlier studies of Meena Nair and Peter (1990) and Solaiappan (2002) who reported that bacteria present in panchagavya acted as biocontrol agent. The data obtained on percentage seed germination, seedling length and seedling vigour index in different treatments are given in table 4. There was significant variation between treatments with respect to percentage seed germination. On 8thday after sowing, significantly highest germination (99%) was noticed in the seeds treated with bacterial isolate BJ5 followed by BJ7 while significantly lowest germination was recorded in uninoculated

Table 4. Germination percentage, seedling length and vigour index of soybean seeds as influenced by inoculations of different bacterial cultures isolated from beejamrutha

Sl. No.	Treatments	Germination %	seedling length	Seedling Vigour index
1.	T_1- Inoculated with BJ1	95	14.68	2647
2.	T_2- Inoculated with BJ2	93	14.9	2560
3.	T_3- Inoculated with BJ3	90	14.62	2484
4.	T_4- Inoculated with BJ4	90	15.96	2746
5.	T_5- Inoculated with BJ5	99	17.11	3276
6.	T_6- Inoculated with BJ6	95	14.81	2669
7.	T_7- Inoculated with BJ7	98	17.05	3181
8.	T_8- Uninoculated control	88	10.55	1864
	S Em±	0.579	0.116	25.96
	CD at 1%	1.756	0.352	78.75

seeds which indicate beneficial role of bacterial isolates in promoting seed germination. The findings of present study support this fact and are in conformity with the study of Ramesh and Thirumurugan (2001) who revealed the effect of seed pelleting and foliar nutrition on better growth of soybean.

Significant variation in seedling length was observed due to various inoculations. Among the treatments, seeds inoculated with BJ5 has registered significantly higher seedling length and seedling vigour index while the seedling length and seedling vigour index was markedly lower in control. The IAA and GA production by the bacterial isolates from beejamrutha could have stimulated germination, seedling length and seed vigour in soybean and hence better result came in all inoculated treatments as compared to uninoculated treatment.

This study clearly brought out that beejamrutha contains not only general microflora, but also certain beneficial biochemical groups such as free living N_2-fixers, P- solubilizers and bacteria producing plant growth promoting substances as well as bacteria having biological deterrent activities. Presence of such beneficial microbial biomass and nutrient status might have resulted in improved seed germination, seedling length and seed vigour in soybean indicating beejamrutha as an efficient plant growth stimulant.

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