

Monetary Benefits

- ❖ Results of on farm experiments showed that by application of rhizospheric (*Azotobacter vinelandii* SRIA3) and endophytic (*Azotobacter chroococcum* Avi2) N-fixing bacteria the mean yield advantage of 0.43 t/ha and 0.53 t/ha, respectively could be achieved over farmer's practice irrespective of rice cultivars.
- ❖ These can lead to monetary benefit of Rs. 4000-9500/- per hectare by using these formulations.
- ❖ In addition to this, these formulations have a potential of cutting down approximately 12-25% total N-fertilizer consumption in lowland rice.

Other Benefits

- ❖ *Azotobacter chroococcum* Avi2 and *Azotobacter vinelandii* SRIA3 are capable of fixing nitrogen at an average of 15-20 kg N/ha/year.
- ❖ They help in synthesis of growth regulating phyto-hormones like auxin, cytokinin and gibberellin.
- ❖ They can improve seed germination and enhance crop growth rate.
- ❖ They can also inhibit phytopathogenic bacteria.
- ❖ They help to increase nutrient availability and to restore soil fertility.

Precautions

- ❖ Should always keep the treated plant under shade condition.
- ❖ Should not mix with any kind of chemical insecticide, fungicide, herbicide and fertilizers.
- ❖ Should wear gloves while mixing and using of these bioinoculants in field.
- ❖ Should avoid inhalation, eye and skin contact during the mixing process.
- ❖ Should avoid drink or smoke during the mixing process.
- ❖ Should wash hands thoroughly with soap water before and after accomplishment of work.

Entrepreneurial opportunities

- ❖ The total annual cost of production for 50 tons of *Azotobacter* grown in 5000 square feet area (Rs. 36 lakhs) with an additional instrumental (Rs. 125 lakhs) and operational (Rs. 31.75 lakhs) cost is 156.75 lakhs. If it can be sold @ Rs. 200 /L., one can reap a benefit of Rs. 68.25 lakhs/annum (Table 2).



Fig. 4. Feedback moments from benefitted farmers at Puri and Cuttack, Odisha.

Table 2. Cost analysis of mass production and upscaling of liquid bioinoculant technology.

Sl. No.	Particulars	Amount (Rs. in lakhs)
Input		
A.	Capital investment	
i.	Building excluding cost of site (App. 5000 sq. ft.)	36.00
ii.	Equipment and apparatus	125.00
	Total capital investment	161.00
B.	Operational cost (Variable cost)	
i.	Working capital (Raw materials)	15.00
ii.	Staff salary and labour	13.00
iii.	Electricity	1.00
iv.	Travelling expenses	2.00
v.	Administrative expenses	0.50
vi.	Miscellaneous expenses	0.25
	Total operational cost	31.75
	Total investment	192.75
Output per annum		
A.	Liquid biofertilizer productions = 50,000 L/annum @ Rs. 200/ L	100.00
B.	Net profit (Excluding capital investment)	68.25
C.	Benefit-Cost Ratio	2.15

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Liquid Bioinoculant of Endophytic (*Azotobacter chroococcum*) and Rhizospheric (*Azotobacter vinelandii*) Nitrogen Fixing Bacteria for Rice Crop



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Liquid Bioinoculant of Endophytic (*Azotobacter chroococcum*) and Rhizospheric (*Azotobacter vinelandii*) Nitrogen Fixing Bacteria for Rice Crop

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Nitrogen (N) is deprived in most of the world's soil which would directly hamper the sustainability of crop production. More than 50% of applied chemical N-fertilizer is lost in rice field and also causes negative influences on soil health thereby affecting crop yield and N-use efficiency. N-fixing microbes are one of the possible alternatives of chemical N-fertilizer. *Azotobacter chroococcum* (Avi2), a native endophyte (less prone to environmental factors) has been explored as a potential diazotroph for rice crop. Avi2 was isolated from root of rice variety Swarna and its endophytic nature has been proved through Fluorescence Resonance Energy Transfer (FRET)-based technique. Further, *in vitro* and *in vivo* analyses of Avi2 were done in rice to prove its diazotrophic efficacy. Besides, *A. vinelandii* SRIA3 (free living nitrogen fixer) was isolated from rice rhizosphere (*var.* Khandagiri), and its nitrogen fixing mechanism has been proved *in vitro* and *in vivo* conditions. Finally, liquid formulations of these two microbes were developed after extensive systematic evaluation. Shelf life of the developed formulation is more than twelve months under ambient condition. Continuous evaluations of these formulations were conducted in 30 different locations of farmer's field in Odisha, which revealed that it can save approximately 12-25% of chemical-N without compromising its yield. Chlorophyll imaging study also revealed the efficacy of Avi2 & SRIA3 in terms of quantum yield of photosystem II (PSII). These formulations have potential to reduce input cost and will help to enhance the farmers' income.

Technology Description

1. Technology Name: Liquid Bioinoculant of Endophytic Nitrogen-Fixing Bacteria for Rice crop

- ❖ **Microbe identity:** *Azotobacter chroococcum* (Avi2), culture deposited in NCMR, Pune, India with accession MCC 3432; NCBI accession no.: KP099933.
- ❖ **Constituents of liquid formulation:** Osmo-protectant/preservative (2-5%), adhesive (4%), disinfectant (0.7-0.8%), lubricator (1.5%) and inoculum (2-5%; 10^8 CFU/mL) in N-free media (Exact composition is kept secret for patent purpose).
- ❖ **Shelf-life:** 12 months at ambient temperature.
- ❖ **Target crop and recommended dose:** Rice (500 ml/ha).
- ❖ **Method of application:** Root dip seedling treatment (4-5 hrs) before transplanting.
- ❖ **Target agro-ecological zones/states:** Eastern India/Odisha.
- ❖ **Validation:** The technology has been validated at 30 locations of farmer's fields in Odisha and also validated at NRRI experimental farm for six consecutive seasons (Fig. 1).

Salient Features

This formulation could be used as root dip for seedlings treatment. It could minimize the harmful effect of chemical N-fertilizer in soil flora and fauna *vis à vis* human health. It could save approximately 25% of N without compromising the rice yield. It increases the seedling vigour and plant growth-promotion in rice. It also increases the plant photosynthesis and N-use efficiencies. The grain yield is *at par* with recommended dose of N-fertilizers and ~10.31% more yield over farmer's practice irrespective of rice cultivars.

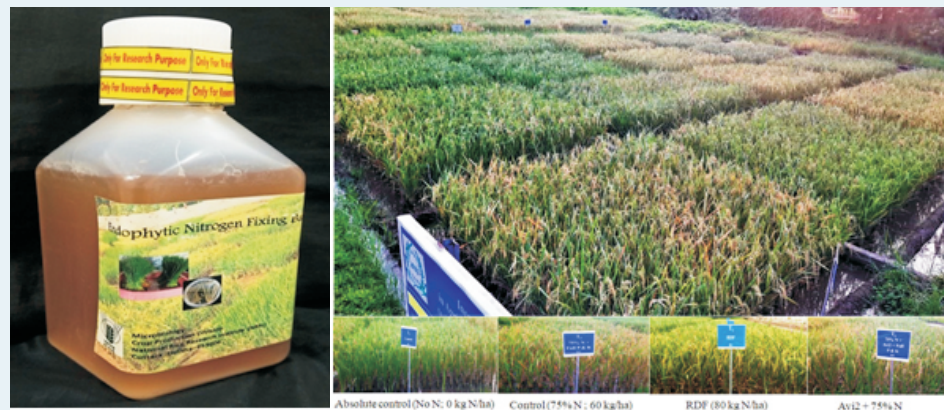


Fig. 1. On station validation of endophytic N-fixing liquid bioinoculant for rice var. Naveen at ICAR-NRRI, Cuttack during 2018-19.

2. Technology Name: Liquid Bioinoculant of Rhizospheric Nitrogen-Fixing Bacteria for Rice crop

- ❖ **Microbe identity:** *Azotobacter vinelandii* SRIAz3, NCBI accession no.: JQ796077.

- ❖ **Constituents of liquid formulation:** Osmo-protectant/preservative (2-5%), adhesive (4%), disinfectant (0.7-0.8%), lubricator (1.5%) and inoculums (2-5%; 10^8 CFU/mL) in N-free media (Exact composition is kept secret for patent purpose).
- ❖ **Shelf-life:** 12 months at ambient temperature.
- ❖ **Target crop and recommended dose:** Rice (500 ml/ha).
- ❖ **Method of application:** Root dip seedling treatment (4-5 hrs) before transplanting.
- ❖ **Target agro-ecological zones/states:** Eastern India/Odisha.
- ❖ **Validation:** The technology has been validated at 30 locations of farmer's fields in Odisha and also validated at NRRI experimental farm for six consecutive seasons (Fig. 2).

Salient Features

This formulation could be used as root dip for seedlings treatment. It could minimize the harmful effect of chemical N-fertilizer in soil flora and fauna *vis à vis* human health. It could save approximately 12-25% of N without compromising the rice yield. It increases the seedling vigour and plant growth-promotion in rice. It also increases the plant photosynthesis and N-use efficiencies. The grain yield is *at par* with recommended dose of N-fertilizers and ~9.22% more yield over farmer's practice irrespective of rice cultivars.

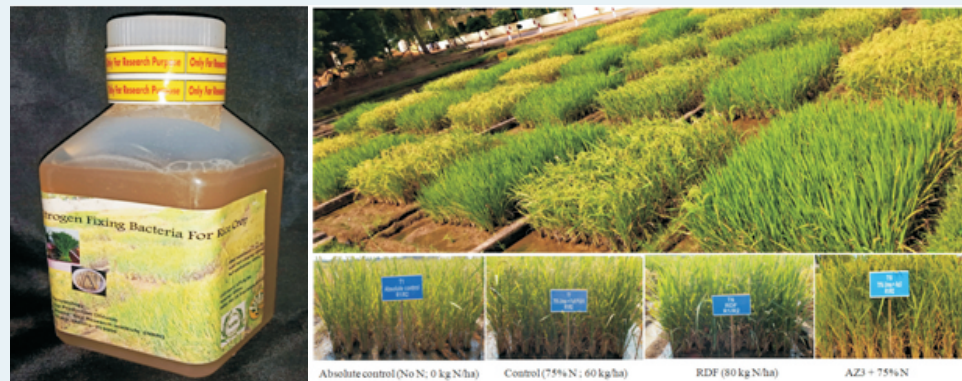


Fig. 2. On station validation of rhizospheric N-fixing liquid bioinoculant for rice var. Naveen and Sabita at ICAR-NRRI, Cuttack during 2017-18.

On Farm Technology Validation

- ❖ On farm evaluations have been conducted at 30 locations of Odisha for two consecutive seasons with different rice cultivars which revealed that these formulations could save ~12-25% chemical N-fertilizer over recommended dose of N-fertilizer and increase the yield by 9.22-10.31% over farmer's practice (Table 1).

Table 1. On farm validation of liquid bioinoculant of endophytic and rhizospheric nitrogen fixing bacteria in rice.

Formulation name	Target rice variety	Number of locations	Yield*	Benefit* (Saving N-fertilizer)
<i>Azotobacter chroococcum</i> Avi2 (Endophytic bacteria)	Sarala	4	<i>At par</i>	~25% N
	Swarna sub 1	6	<i>At par</i>	~25% N
	Hasanta	4	<i>At par</i>	~25% N
	1009 sub1	4	<i>At par</i>	~25% N
	Bina 11	4	<i>At par</i>	~25% N
	Lalat	4	<i>At par</i>	~25% N
<i>Azotobacter vinelandii</i> SRIAz3 (Rhizospheric bacteria)	Sarala	4	<i>At par</i>	12- 25% N
	Swarna sub 1	6	<i>At par</i>	12- 25% N
	Hasanta	4	<i>At par</i>	12- 25% N
	1009 sub1	4	<i>At par</i>	12- 25% N
	Bina	4	<i>At par</i>	12- 25% N
	Lalat	4	<i>At par</i>	12- 25% N
Khandgiri	4	<i>At par</i>	12- 25% N	

*As compared to recommended dose of N.

Impacts of Technology

- ❖ These two liquid bioinoculants technologies have been widely disseminated through awareness and hands on training *cum* demonstration programme among different stakeholders of Odisha (Fig. 3).
- ❖ Impact analysis has been made by taking feedback from 25 beneficiary and 13 non-beneficiary farmers across the targeted sites which showed that these formulations could enhance yield by 9.22 - 10.31% and save ~12-25% of chemical N (Fig. 4).



Fig. 3. Farmers awareness *cum* demonstration programme on endophytic and rhizospheric nitrogen fixing liquid bioinoculant for rice at Puri and Cuttack, Odisha during 2018-19.