APPENDIX – IV

भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS

Doc. No.: PRTD/AR/PF:03	Issue No.: 2	Issue Date 30 Sept. 2020	**Report of Action Research**
1.	**Action Research Project No. (as assigned by PRTD)**		AR/0132
2.	**Title of the Action Research Project**		Revision/Amendmentof existing standard IS 14806:2000 Azospirillum inoculants
3.	**Name & Designation of Officer**		Prem Sajani Patnala, Scientist-D
4.	**Employee No.**		063444
5.	**Deptt./BO/RO & Place of Posting**		BNBO, BANGALORE
6.	**Date of Approval of the Project**		17 Dec 2020
7.	**Objective of the Project**		To revise/amend the existing standard IS 14806:2000, Azospirillum inoculants, though reaffirmed in 2021, as few errors were observed while reviewing, because of which the following action research was proposed.
8.	**Report of Action Research Activities**		Attached as Annexure 3-13
9.	**Conclusion & Recommendations**		IS 14806: 2000 has to be revised as there are so many typographical errors.
10.	**Any other relevant information**		NIL

Prem Saj 28/4/2021
Sign. of Officer (Prem Sajani)

Head of Deptt./BO 27/04/2021
DDGS
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INTRODUCTION

The bacteria of genus Azospirillum is gram negative, microaerophilic and fixes nitrogen in the roots of many non-leguminous crops like cereals, millets, forage crops and vegetables, etc. Besides fixing nitrogen, it also produces growth promoting substances required for vegetative growth of the plants. These bacteria although present in most soil types, the number of Azospirillum and extent of the benefits varies in the soil.

Azospirillum is considered as very important diazotrophs as it forms associative symbiotic relationship with the roots of graminaceous plants. It is generally recommended for rice crop. The organism is microaerophillic, some are aerobic, motile and gram negative in nature hence suits well for rice field conditions. It was first isolated by Beijernick and was named as Spirillum lipoferum later named as Azospirillum. In addition to nitrogen fixing ability, they also produce growth promoting substances such as IAA. Some of the important species of Azospirillum are A. brasilense, A. lipoferum, A. amazonense, A. halopraeferens, A. irkense, A. dobereinerae, A. largimobilis

Many brands of Azospirillum inoculants are marketed in the country and they have been found to vary in quality. It is obligatory on the part of the manufacturers to employ qualified Soil Microbiologist Agricultural Graduates/Graduates in Biology trained in soil microbiology in their staff. Manufacturers shall also maintain a quality control laboratory capable of carrying out the tests according to this specification.

REVIEW OF LITERATURE

Bio fertilizers nowadays have been realised for shifting fortunes in agriculture. It has been proven successful technology in many developed countries while in developing countries exploitation of bio inoculants is hampered by several factors. Scientific knowledge on bio inoculants and its usage will pave way for their effective usage. At the same time overlooking the significance of ensuring and maintaining a high-quality standard of the product will have a negative impact. Hence a proper knowledge of bio inoculants and its functioning will pave the way to tape the resources in a better way.

Bio fertilizers are microbial inoculants which can be usually defined as a preparation containing live or dormant cells of efficient strains of nitrogen-fixing, phosphate solubilizing, and cellulytic microorganisms, etc. A bio fertilizer utilizes living microorganisms to improve the growth of seeds and plants. They are typically applied into the soil where they colonize the rhizosphere or the interior of the plant and aid the supply or accessibility of nutrients. In nature, there are several useful soil microorganisms which can help plants to absorb nutrients. Their utility can be enhanced with human intervention by selecting efficient organisms, culturing them and adding them to soils directly or through seeds. The cultured microorganisms packed in some carrier material for easy application in the field are called bio-fertilisers. Thus, the critical input in Bio fertilizers is the microorganisms.

ADVANTAGES

Bio fertilizers restore normal fertility to the soil and make it biologically alive. They boost the amount of organic matter and improve soil texture and structure. The enhanced soil holds water better than before. They also help to

1. achieve higher crop yields while also enhancing soil health
2. replace chemical fertilizers which are not beneficial for long term plant health, and can be toxic to the environment and consumers
3. may be performed as a by-product of electricity generation from biogas
4. combat pathogens in both soil and plant, and work as a natural pesticide
5. relatively easy to apply
6. combat the effects of drought and other restrictive conditions
7. are cost effective, and are affordable even for low-income farmers
8. Bio fertilizers fix atmospheric nitrogen in the soil and root nodules of legume crops and make it available to the plant.
9. They solubilise the insoluble forms of phosphates like tricalcium, iron and aluminium phosphates into available forms.
10. They scavenge phosphate from soil layers.
11. They produce hormones and anti-metabolites which promote root growth.
12. They decompose organic matter and help in mineralization in soil.
13. When applied to seed or soil, bio fertilizers increase the availability of nutrients and improve the yield by 10 to 25% without adversely affecting the soil and environment.

DISADVANTAGES

There are few disadvantages also which are listed below

1. Requires specific machinery
2. require special care for long-term storage because they are alive. They must be used before their expiration date.
3. Provide lower nutrient density than chemical fertilizers so more product is often required for the same effect
4. If other microorganisms contaminate the carrier medium or if growers use the wrong strain, they are not as effective. The soil must contain adequate nutrients for bio fertilizer organisms to thrive and work.
5. complement other fertilizers, but they cannot replace them.
6. lose their effectiveness if the soil is too hot or dry. Excessively acidic or alkaline soils also hamper successful growth of the beneficial microorganisms; moreover, they are less effective if the soil contains an excess of their natural microbiological enemies.

TYPES AND FEATURES OF BIOFERTILIZERS

Based on type of microorganism, the bio-fertilizer can also be classified as follows:

- Bacterial Bio fertilizers: e.g. Rhizobium, Azospirilium, Azotobacter, Phosphobacteria.
- Fungal Bio fertilizers: e.g. Mycorhiza
- Algal Bio fertilizers: e.g. Blue Green Algae (BGA) and Azolla. *
- Actinimycetes Biofertilizer: e.g. Frankia.

*Bio-fertilizer are mostly cultured and multiplied it the laboratory. However, blue green algae and azolla can be mass-multiplied in the field. Soil, rock phosphate pellet, paddy straw compost, wheat bran, or a mixture of such materials, etc. provides better shelf life to bio fertilizer formulation.

BIOLOGICAL NITROGEN FIXATION

Biological nitrogen fixation is a component of nitrogen cycle which involves fixing up of atmospheric nitrogen by particular soil microorganisms. Nitrogen fixing ability has been restricted only to certain bacteria and few actinomycetes which belong to various groups and are referred to as diazotrophs. Diazotrophic microbes are ubiquitous to soil and are classified according to mode of nitrogen fixation to plants. They can be grouped in different ways based on their nature and function

SYMBIOTIC NITROGEN-FIXING BACTERIA Examples are Rhizobia which are diazotrophic bacteria that fix nitrogen after becoming established inside the root nodules of legumes (Fabaceae). To express genes for nitrogen fixation, rhizobia require a plant host; they cannot independently fix nitrogen.

1. LOOSE ASSOCIATION OF NITROGEN-FIXING BACTERIA

Azospirillum is a nitrogen-fixing bacterium that live around the roots of higher plants but do not develop an intimate relationship with plants. It is often termed as rhizosphere association as this

bacterium collect plant exudate and the same is used as a food by them. This process is termed as associative mutualism.

2 SYMBIOTIC NITROGEN-FIXING CYANOBACTERIA

Blue-Green algae or Cyanobacteria from the symbiotic association with several plants. Liverworts, cycad roots, fern, and lichens are some of the Nitrogen-fixing cyanobacteria. Anabaena is found at the leaf cavities of the fern. It is responsible for nitrogen fixation. The fern plants decay and release the same for utilization of the rice plants. Azolla pinnate is a fern that resides in rice fields but they do not regulate the growth of the plant.

3. FREE-LIVING NITROGEN-FIXING BACTERIA

They are free-living soil bacteria which perform nitrogen fixation. They are saprotrophic anaerobes such as Clostridium beijerinckii, Azotobacter, Klebsiella, Anabaena, Nostoc etc.

METHODS & MATERIALS

1. A visit was paid to **M/s MULTIPLEX BIOTECH PVT LTD,(CM/L- 6681181)** our Licensee for IS 14806:2000 where inputs were taken from Dr.Nagaraj.

A visit was paid to Azospirillum inoculant manufacturing unit name M/s MULTIPLEX BIOTECH PVT LTD who is currently a licensee at the BIS, Bangalore Branch Office. The Purpose of the visit to better understand the market perspective of Azospirullum the demand for the same, its application and consumer base. During the course of visit the manufacturer also shared how they implement the ISS to maintain the quality and conformity of the product.

2. A visit was also made to **Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK campus, Bangalore-560065 where Dr.M.K.ShivaPrakash, ICAR-Emeritus Professor** was kind enough to share his experiences and inputs to the standard

Suggested efficiency character is very important to analyse the efficiency of bio fertilizer used. Also was of the opinion that, bio fertilizers main efficiency lies in number of viable cells present in the carrier material at the time of use and number of viable cells keep on reducing over a period of time due to non-availability of proper growth conditions. Hence shelf life to be limited to six months

3. Also inputs taken from Dr.Mohanraj, **Shivashakthi Biotechnologies Limited, Sy.no 170/A & AA, Anantaram Village, Medak, Jinnaram Mandal,Telangana**-502319 via email and telephonic conversation

4. Inputs from **Cm/L- 8200121797, INDORE BIOTECH INPUTS AND RESEARCH PVT LTD, Gram Dehari, Khasara No.204/3, Rau-Pithampur Road, Opp. IIM, Rangwasa, INDORE- 452001** received via email and telephonic conversation.

   a.) Regarding expiry, firm informed that test for effective Nodulation requires minimum 3 months for the completion, but if we fix expiry for only 6 months it is not practically possible and hence it shall be minimum 6 months from the date of manufacture.

   b.) For the moisture percentage, firm wants to use dextrose as a carrier, in which case 30-40% moisture percentage changes as it depends on carrier.

OTHER STANDARDS

- No ISO standards/other international standards available for Azospirillum
- Standard contains no Cross Referenced International Standard.
- Standard contains no Cross Referenced Indian Standard.
- All manufacturers of Biofertilizers in India, have to comply with No.11-3/83-STU Government of India Ministry of Agriculture and Rural Development (Department of Agriculture and Cooperation) New Delhi, dated 25th September 1985, THE FERTILISER (CONTROL) ORDER 1985 as per Government of India

THE FERTILISER (CONTROL) ORDER 1985

A comparison has been made with their requirements and Indian Standard requirements.

	AS PER THE FERTILISER (CONTROL) ORDER 1985 (as amended up to Feb 2019)	**AS PER IS 14806:2000**	**Remarks**
	(i) Base = Carrier based* in form of moist/dry powder or granules, or liquid based	Only carrier based moist/dry powder or granules	Liquid based may also be included as it is already allowed as per FCO and Licensee also is manufacturing and supplying in Liquid state as few customers are requesting as they found liquid based is more effective.
	(ii) Viable cell count = CFU minimum $5x10^7$ cell/g of powder/granules or carrier material or 1 $x10^8$ cell/ml of liquid	cl.4 ASI shall contain 107* viable Azospirillum cells/g of the carrier material on dry mass basis *Actually must be 10^7 Typographical error in the existing standard	Firm is maintaining anyway 5 x 107 cells/g of powder as per FCO orders. Hence our requirements may also be increased to match FCO guidelines.
	(iii) Contamination level = No contamination at 10^5 dilution	4.3 ASI 'when tested by the method prescribed in Annex A, shall have no contamination with other micro-organisms at 10^5 dilution	No change required
	(iv) pH = 6.5 – 7.5	Cl 4.4 pH- 6.5 to 7.5	No change required
	(v) Particle size in case of carrier-based material = All material shall pass through 0.15-0.212 mm IS Sieve	cl.4.2 and Amendment no.1- '150 to 212 μ (72 to 100 mesh)	No change required

	(vi)Moisture percent by weight, maximum in case of carrier based = 30-40%	Cl 4.5 Moisture 30-40%	No change required
	(vii) Efficiency character = Formation of white pellicle in semisolid Nitrogen free bromothymol blue media	---	May also be included as efficiency character is very important to analyse the efficiency of biofertilizer used. Licensee and Dr. M. K. Shiva Prakash, ICAR-Emeritus Professor also suggested the same. Licensees calculating it as per FCO order as well as to know efficiency of their final product.
	*Type of carrier: The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of the organism.	The powder carrier material such as peat, lignite, charcoal or similar material such as soil: cow dung or soil: compost may be used. It shall be neutralized with calcium carbonate and then sterilized	No change required

RECOMMENDATIONS AND CONCLUSIONS

Based on the detailed study the following observations made. The following changes as proposed, may be incorporated in the Standard and Amendment or revised Standard may be released.

S.no	IS 14806: 2000	Existing	Needs to be amended as	JUSTIFICATION
1	Page no.1 clause 4	ASI shall contain 107 viable Azospirillum cells/g of the carrier material on dry mass basis	ASI shall contain **5X10^7** viable Azospirillum cells/g of the carrier material on dry mass basis	In similar standards the requirements for the same parameter are 5X10^7/g (IS 9138:2020) 5 X 10^7 /g (IS 14807:2021) 5 X 10^7/g (IS 8268:2020) Serial dilutions will always be in "10 to the power of n" denominations As per FCO guidelines, must be 5 x 10^7/g
2	Page no.1, cl 4.3	4th line 'Semi' dilution	'**Serial**' dilution	Serial dilution is the more appropriate technical word
3	Page no.1, cl 4.3, 5th and 6th line	ASI contamination in semi solid medium should be checked by semi dilution and spread plate method with solid complete medium	.....dilution and spread plate method with **malate semi solid medium or malate agar medium**	Malate semi solid medium or malate agar medium is specific medium for Azospirillum sp.
4	Page no.1 cl.4.7	It was mentioned that "The manufacturer may control the quality of the broth as given in Annex D, it should get verified at least by two institution"	Cl.4.7 & note under it may be reworded as "**Specified mother culture be obtained from any recognized institution maintaining the mother cultures.** The manufacturer may control the quality of the broth as given in Annex D."	For a manufacturer it is not possible to get each control unit of broth verified from 2 different institutions as given in Cl.4.7 & note under it. May be modified as given in other similar ISS 14807, IS 8268 and IS 9138.
5	Page no.1 Clause 4.5	In cl.4.5 requirement for moisture, was prescribed but no test method mentioned. But in page no.3, Annex B it was mentioned as	In page no.1 **reference to Annex B** shall be given in Cl 4.5. In Annex B it should be mentioned as for **Clause 4.4 & 4.5**	TYPOGRAPHICAL ERROR

		for Determination of P^H and Moisture percentage		
6	In page no.3, Annex C	it was mentioned as Clause 4.5. But in Page no.1 Clause 4.5 is for moisture requirement.	In page no.3 Annex C should be corrected as **Clause 4.6**	TYPOGRAPHICAL ERROR
7	In page no.2, Annex A,A-3.4, 3rd line 4TH line 9th line	serial dilutions up to 107 dilution aliquots of 105 to 107 dilution 105,106 & 107 level	It should be corrected as serial dilutions up to **10^7** dilution aliquots of **10^5 to 10^7** dilution **10^5,10^6 & 10^7** levels	Serial dilutions will always be in "10 to the power of n" denominations
8	In page no.3 Annex C, C-2, d)	Pipette 1 ml of each dilution (from 10-1 to 10-7) to each one of 5 replicates	d) Pipette 1 ml of each dilution (from **10^{-1} to 10^{-7}**) to each one of 5 replicates	Serial dilutions will always be in "10 to the power of n" denominations
9	In page no.3 Annex C, C-2	f.) 105,106 and 107 dilution level	It should be corrected as **10^5,10^6 and 10^7** dilution level	Serial dilutions will always be in "10 to the power of n" denominations
10	Page no.5 Annex E	E-2 Azospirillum brasiliense	The correct nomenclature is Azospirillum **brasilense**	TYPOGRAPHICAL ERROR
11	Page no.2 cl.6.1g	Expiry date which shall not be less than 6 months from the date of manufacture	It should be Expiry date which shall not be **more** than 6 months from the date of manufacture	Biofertilizers main efficiency lies in number of viable cells present in the carrier material at the time of use. Number of Viable cells keep on reducing over a period of time due to non-availability of proper growth conditions. Hence shelf life to be limited to six months. (Also one of the point been suggested by Dr. M.K. Shiva Prakash, ICAR-Emeritus Professor) But our Licensees M/s INDORE BIOTECH

				INPUTS AND RESEARCH PVT LTD is of the opinion that test for effective Nodulation requires minimum 3 months for the completion, but if we fix expiry for only 6 months it is not practically possible and hence it shall be minimum 6 months from the date of manufacture and M/s MULTIPLEX BIOTECH PVT LTD was of the opinion that expiry and viability depends on the strain used hence shelf life can be minimum six months.
12	---	---	**Efficiency character** may also be included as efficiency character is very important to analyse the efficiency of biofertilizer used.	(Suggested by Licensee as well as Dr. M.K. Shiva Prakash, ICAR-Emeritus Professor and as per FCO)
13	---	---	**Liquid based also may be allowed in the standard.**	Liquid based may also be included as it is already allowed as per FCO and Licensee also is manufacturing and supplying in Liquid state as few customers are requesting as they found liquid based is more effective

DETAILS OF THE BIS SUPPORT AVAILED

Transport arranged to visit

1. M/s MULTIPLEX BIOTECH PVT LTD,(CM/L- 6681181)
2. Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK campus, Bangalore

REFERENCES

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2. Booklet on Bio fertilizers - Phosphobacteria
3. TNAU Agritech portal
4. NABARD Knowledge bank
5. Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms, Mateusz MącikAgata GrytaMagdalena Frąc, in Advances in Agronomy, 2020
6. No.11-3/83-STU Government of India Ministry of Agriculture and Rural Development (Department of Agriculture and Cooperation) New Delhi, dated 25th September 1985 THE FERTILISER (CONTROL) ORDER 1985