*भारतीय मानक*

***Indian Standard***

**IS 18363 : 2023**

# ज़िंक घुलनशील बैक्टीरियल इनोकुलेंट (ज़ेडएनएसबीआई) — विशिष्टि

# Zinc Solubilizing Bacterial Inoculant (ZnSBI) — Specification

ICS 65.080



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**October 2023** **Price Group 5**

Soil Quality and Fertilizers Sectional Committee, FAD 07

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soil Quality and Fertilizers Sectional Committee had been approved by the Food and Agriculture Division Council.

Biofertilizers have been identified as alternative to chemical fertilizers to increase soil fertility and crop production in sustainable farming. Biofertilizers are natural fertilizers which are microbial inoculants of bacteria, algae, fungi alone, or in combination, and they augment the availability of nutrients to the plants. The use of biofertilizers, in preference to chemical fertilizers, offers economic and ecological benefits by way of soil health and fertility to farmers.

Biofertilizers are products containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available form through biological processes. They are involved in symbiotic and associative microbial activities with higher plants. These are natural mini-fertilizer factories that are economical and safer source of plant nutrition for increasing the agricultural production and improving soil fertility.

The zinc solubilizing bacterial inoculants (ZnSBI) include different groups of soil micro-organisms which solubilize insoluble zinc compounds into soluble form. The species of *Bacillus* and *Pseudomonas* are active in bioconversion. The most efficient bacterial isolates are identified as *Bacillus subtilis.* These efficient microorganisms have shown capability to solubilize zinc content available in soil naturally or applied in the soil through fertilizer application.

Many brands of (ZnSBI) are marketed in the country and they have been found to vary in quality. Hence, the need was felt for an Indian Standard not only to test the quality of inoculants in order to provide the farmers with certified inoculants but also to help the producers to improve quality of their products.

The composition of the Committee responsible for the formulation of this standard is given in Annex D.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of test or analysis, shall be rounded off in accordance with IS 2 : 2022 ‘Rules for rounding off numerical values (*second revision*)’. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

*Indian Standard*

# ZINC SOLUBILIZING BACTERIAL INOCULANT (ZnSBI) — SPECIFICATION

## 1 SCOPE

This standard prescribes the requirements method of sampling and tests for zinc solubilizing bacterial inoculants.

## 2 REFERENCES

The standards given below contain provisions which through reference in this text constitutes provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of these standards:

| *IS No.* | *Title* |
|---|---|
| IS 460 (Part 1) : 2020 | Test sieves — Specification: Part 1 Wire cloth test sieves (*fourth revision*) |
| IS 1070 : 2023 | Reagent grade water (*fourth revision*) |
| IS 8268 : 2020 | *Rhizobium* inoculants — Specification (*third revision*) |

## 3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

**3.1.1** *Zinc Solubilizing Bacterial lnoculant (ZnSBI)* — ZnSBI is a product having high population of a strain(s) of bacteria intended to solubilize unavailable zinc in the soil to the plant usable soluble form.

**3.1.2** *Group of Crops* — ZnSBI is beneficial for all types of crops in terms of solubilization of zinc present in the soil in insoluble form.

## 4 REQUIREMENTS

**4.1** ZnSBI shall be carrier based and the colour depending on the colour of the carrier. Carrier material such as peat, lignite, charcoal or similar material may be used. In case if carrier is acidic in nature then it shall be neutralized with calcium carbonate and then sterilized. When tested for fineness it shall pass through 0.15 mm to 0.212 mm IS sieve [*see* IS 460 (Part 1)].

**4.2** When tested by the method prescribed in Annex A, ZnSBI shall contain a minimum of $5 \times 10^7$ viable zinc solubilizing bacterial cells/g of the carrier material on dry mass basis during the entire period of shelf life, as specified on the packet.

**4.3** When tested by the method prescribed in Annex A of IS 8268, ZnSBI shall have no contamination with other microorganisms at $10^5$ dilutions.

**4.4** When tested by the method prescribed in Annex B of IS 8268, the *p*H of ZnSBI shall be 6.5 to 7.5.

**4.5** When tested by the method prescribed in Annex A, ZnSBI shall show minimum of 10 mm solubilization zone in prescribed media having at least 3 mm thickness.

**4.6** Specified mother culture be obtained from any recognized institution maintaining the mother culture. The manufacturer may control the quality of broth as given in Annex B, it should get verified at least by two institutions as mentioned below.

NOTE — At present *National Centre of Organic Farming* (*NCOF*), Ghaziabad and its six Regional Centres located at Bangalore, Bhubaneshwar, Hissar, Imphal, Jabalpur and Nagpur; Indian Agricultural Research Institute (IARI), New Delhi; Tamil Nadu Agricultural University (TNAU), Coimbatore; University of Agricultural Science, Bangalore are sources for supplying the mother culture.

**4.7** The ZnSBI carrier shall be friable (moist) with 30 percent to 40 percent (*w/v*) moisture content. Moisture content shall be determined as given in Annex B of IS 8268.

## 5 PACKING, MARKING AND STORAGE

### 5.1 Packing

ZnSBI shall be packed in packaging material of low density polyethylene/polypropylene bags thickness of which shall not be less than 75 micron to 100 micron or in suitable HDPE/polypropylene/ glass bottles.

### 5.2 Marking

**5.2.1** Each polyethylene pack shall be marked legibly and indelibly with the following information:

a) Name of the product specially as 'ZINC SOLUBILIZING BACTERIAL INOCULANT';
b) Name and address of the manufacturer;
c) Crop(s) for which intended;
d) Type of the carrier;
e) Batch number;
f) Date of manufacture;
g) Expiry date which shall not be less than 6 months from the date of manufacture;
h) Net quantity in kg and area meant for;
j) Storage instructions worded as under 'STORE IN COOL PLACE AWAY FROM DIRECT SUNLIGHT AND HEAT'; and
k) Any other information required under the *Legal Metrology* (*Packaged Commodities*) *Rules*, 2011.

**5.2.2** Items (c), (f) and (g) shall be printed on a coloured ink background.

**5.2.3** Directions for use of ZnSBI shall be printed briefly on the packets as given in Annex C of this standard. A separate pamphlet may preferably be given with it.

**5.2.4** *BIS Certification Marking*

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

### 5.3 Storage

ZnSBI shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 20 ºC and not exceeding 30 ºC. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.

## 6 SAMPLING

The representative samples of the ZnSBI shall be drawn as prescribed in Annex F of IS 8268.

## 7 TESTS

**7.1** Tests shall be carried out by the methods prescribed in **4**.

**7.2** Unless otherwise specified, quality reagents, chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect experimental results.

# ANNEX A

(*Clauses* 4.2, 4.3 *and* 4.5)

## DETERMINATION OF NUMBER OF ZINC SOLUBILIZING BACTERIAL CELLS

### A-1 APPARATUS

**A-1.1 Pipettes** — Graduated, 1 ml and 10 ml

**A-1.2 Conical Flasks** —150 ml and 250 ml

**A-1.3 Screw Capped Tubes** —10 ml

**A-1.4 Incubator**

**A-1.5 Petri Dishes** — Clear, uniform, flat-bottomed

**A-1.6 Hot Air Oven**

**A-1.7 Autoclave**

**A-1.8 *p*H Meter**

**A-1.9 Colony Counter**

**A-1.10 Hand Tally or Mechanical Counting Device**

**A-1.11 Magnifying Lens**

### A-2 REAGENTS

**A-2.1** Use a medium of the following composition for analysis of total viable count and contamination:

| | |
|---|---|
| Glucose | 10 g |
| Zinc oxide | 1.0 g |
| Ammonium sulphate | 0.5 g |
| Potassium chloride | 0.2 g |
| Yeast extract | 0.5 g |
| Ferrous sulphate | 0.01 g |
| Manganese sulphate | 0.01 g |
| Di potassium hydrogen phosphate | 0.5 g |
| Distilled water | 1 000 ml |

Mix all the ingredients in about 750 ml of water, except glucose and autoclave. Filter sterilized glucose, dissolve in 250 ml water and mix with above solution after autoclaving.

**A-2.2** Sterilization and preparation process for plates is the same as described in IS 8268.

### A-3 PREPARATION OF SERIAL DILUTION FOR CELL COUNT METHOD

Dispense 30 g of ZnSBI in 270 ml of sterile water and shake for 10 minutes on a reciprocal shaker. Make serial dilutions up to $10^7$ level. Pipette out 0.2 ml aliquots of $10^5$ to $10^7$ dilution and deliver it on the petri dishes containing set medium as described in **A-2.1**. Spread the aliquots over the plate. Invert the plates and place them in the incubator at 28 °C ± 2 °C for 7 days. Use 3 replicates of $10^5$, $10^6$ and $10^7$ dilution.

### A-4 COUNTING

Count total number of colonies on the plates including colonies with solubilization zone with the help of a colony counter.

### A-5 METHOD FOR SOLUBILIZATION ZONES

**A-5.1** Take 10 g of ZnSBI in 90 ml in water.

**A-5.2** Make ten folds dilution series up to $10^{10}$ dilutions.

**A-5.3** Take 0.2 ml aliquots of $10^5$ to $10^7$ dilution using sterile pipettes and delivered to petri dishes containing Aleksandrov medium.

**A-5.4** Spread it uniformly. Invert the plates and incubate them up to 2 weeks at 28 °C ± 2 °C.

**A-5.5** Count the colonies showing hollow zones and measure their diameter. Minimum acceptable zone is 10 mm in diameter.

## ANNEX B

(*Clause* 4.6)

## GUIDELINES ON MAINTENANCE AND PREPERATION OF CULTURE AND QUALITY CONTROL AT BROTH STAGE

### B-1 MAINTENANCE OF PURE CULTURES

**B-1.1** Maintain pure culture of ZnSBI on the medium as described in **A-2.1** in the form of slants.

**B-1.2** Transfer a loopful of pure culture to each of the agar slants aseptically in an inoculation room and incubate at 28 °C ± 2 °C for 7 days. Always keep pure culture below 5 °C.

### B-2 PREPARATION OF INOCULUM CULTURE AND MASS CULTURE

Inoculum culture and mass culture shall be prepared as described in **D-2.1**, **D-2.2** and **D-3** of the IS 8268 changing the media composition as mentioned in **A-2.1** of this standard.

### B-3 QUALITY CONTROL TEST RECOMMENDED AT BROTH STAGE

#### B-3.1 Qualitative Test

**B-3.1.1** Check for freedom from visible contaminants by microscope and observing solubilization zones.

**B-3.1.2** *p*H of bacterial broth shall normally be between 6.5 to 7.

**B-3.1.3** Gram staining test shall be carried out as described in **D-4.1.3** of IS 8268.

#### B-3.2 Quantitative Test

**B-3.2.1** *Viable Cell Count*

As per the method mentioned at Annex A of this standard.

## ANNEX C

(*Clause* 5.2.3)

## DIRECTION FOR USE OF ZINC SOLUBILIZING BACTERIAL INOCULANTS

**C-1** The contents of the packet are sufficient enough to the given quantity of seed for seed treatment or for given area to be broadcast or for given quantity of seedlings for root dipping depending on the specified crops as denoted on the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. Dry the inoculated seed under shade over clean dry paper or organic bag and sow immediately. For seedling treatment suspend recommended quantity of inoculants in recommended quantity of water and keep the roots of seedlings dipped for 30 min to 45 min immediately before transplanting. For soil treatment mix recommended quantity of inoculants in 200 kg to 500 kg of mature compost and broadcast evenly over recommended area at the time of final ploughing before sowing.

**C-2** In order to mobilize unavailable soil zinc use ZnSBI in all type of crops. Use before the expiry date and do not expose to direct sunlight or heat.

**C-3** ZnSBI is not a chemical fertilizer, hence do not mix inoculated seeds or ZnSBI with agro-chemicals.

## ANNEX D

(*Foreword*)

## COMMITTEE COMPOSITION

Soil Quality and Fertilizers Sectional committee, FAD 07

| *Organization* | *Representative(s)* |
|---|---|
| ICAR - Natural Resource Management Division, New Delhi | DR S. K. CHAUDHARI **(*Chairperson*)** |
| Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur | PROF B. MANDAL<br>PROF G. C. HAZRA (*Alternate*) |
| CCS Haryana Agricultural University, Hisar | HEAD (SOIL SCIENCE)<br>HEAD (MICROBIOLOGY) (*Alternate*) |
| Central Fertilizers Quality Control and Training Institute, Faridabad | SHRI SHYAM BABU |
| Central Soil Salinity Research Institute, Karnal | DR A. K. RAI<br>DR ASHIM DATTA (*Alternate*) |
| Chambal Fertilizers and Chemicals Limited, New Delhi | DR NARESH PRASAD<br>DR A. K. BARANWAL (*Alternate*) |
| Consumer Guidance Society of India, Mumbai | DR SITARAM DIXIT |
| Fertilizer Association of India, New Delhi | DR R. K. TEWATIA |
| G. B. Pant University of Agriculture and Technology, Pantnagar | DR ANIL K. SHARMA |
| Gujrat State Fertilizer and Chemicals Limited, Vadodara | SHRI V. N. GOTHI<br>SHRI K. G. KORANT (*Alternate*) |
| Indian Agricultural Research Institute, New Delhi | HEAD (SOIL SCIENCE)<br>HEAD (MICROBIOLOGY) (*Alternate*) |
| Indian Farmer Fertilizer Cooperative Limited (IFFCO), New Delhi | DR RADHA KRISHNAN NAYAK |
| Indian Institute of Soil Science, Bhopal | DR ASHOK K. PATRA<br>DR M. C. MANNA (*Alternate*) |
| Indian Micro Fertilizers Manufacturers Association, Pune | SHRI MAHESH G. SHETTEY |
| International Panacea Limited, New Delhi | DR RANVIR SINGH<br>SHRIMATI VIMALA PRAKASH (*Alternate*) |
| IRC Agrochemical, Kolkata | DR B. B. SINGH<br>SHIR BIDHAN SINHA (*Alternate*) |

| *Organization* | *Representative(s)* |
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| National Fertilizer Limited, Noida | DR JYOTI GOEL<br>SHRI PREMLAL (*Alternate*) |
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| Potash Research Institute of India, Gurugram | SHRI S. K. BANSAL |
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| National Bureau of Agriculturally Important Microorganism (NBAIM), Mau Nath Bhanjan | DR ANIL KUMAR SAXENA<br>DR ALOK KUMAR SRIVASTAVA(*Alternate*) |
| Yara Fertilizer India Private Limited, Jalgaon | DR VIPIN MISHRA |
| BIS Directorate General | SHRIMATI SUNEETI TOTEJA, SCIENTIST 'E'/ DIRECTOR AND HEAD (FOOD AND AGRICULTURE) [REPRESENTING DIRECTOR GENERAL (*Ex-officio*)] |

*Member Secretary*
SHRI RAJPAL
SCIENTIST 'D'/JOINT DIRECTOR
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