ETHIOPIAN STANDARD

ES 3907-1: 2015

Fertilizers-Biofertilizers-part 1:-Rhizobial specification and test method.

ICS: 65.080

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Foreword

This Ethiopian Standard has been prepared under the direction of Technical Committee for **Fertilizer** (TC 15) and published by the Ethiopian Standards Agency (ESA).

The draft document (Working D raft, WD) has been submitted t o t he Secretariat by the Ethiopian Institute of Agricultural Research/EIAR/.

References

During the preparation of this standard reference was made to the following documents:

EAS 456: 2007- East African Organic Products Standard

The FNCA biofertilizer manual 2006

Indian bio fertilizer standards-

IS 8268:2001-Standard for Rhizobium

IS 9138:2002-standard for Azotobacter

IS 14806:2000-Standard for Azospirillum

IS 14807:2000-Standard for phosphate solubilizers

Acknowledgement has been made to the said organization for their effort of presenting the draft document and for the assistance derived from the above sources.

Introduction

Fertilizers directly increase soil fertility by adding nutrients. Biofertilizers add nutrients through the natural processes of fixing atmospheric nitrogen, solubilizing Phosphate or nutrient mobilization to stimulate plant growth through the synthesis of growth promoting substances. They can be grouped in different ways based on their nature and function. Most biofertilizers are produced from microorganisms such as *Rhizobium*, Azotobacter, Azospirillum, Phosphate solubilising bacteria. Other types include mycorrhizal biofertilizers, potassium mobilizing biofertilizers and zinc solubilising biofertilizers.

The use of biofertilizers offer economic and ecological benefits by way of soil health improvement and fertility. This standard is in line with the fertilizer policy of Ethiopia which aims to ensure availability of high quality biofertilizer products for efficient use by the farmers

Regulations on inoculant quality vary from country to country and no set of international standards exists. Brazil, Canada, France, and Uruguay have regulatory authorities supported by legislation. Australia, South Africa, and New Zealand have quality control programs in which inoculant manufacturers participate voluntarily. In many other countries such as USA and UK product quality standards are left to the discretion of the manufacturers. Whether legislatively set or internally established by the manufacturer, standards for inoculants products should be a compromise between theoretical possibilities and practical limitations. However, without defined standards quality control cannot work (Thompson, 1991a). There exists a consensus that the establishment of standards, whether voluntary or imposed, has improved legume inoculant quality.

The objective of this standard is to ensure that biofertilizers on the market are appropriately tested through the quality criteria provided while ensuring that farmers obtain only certified products and as well aid the industry in the manufacture of quality biofertilizers. This standard will also promote the safe use of bioferilizers and promote fair trade.

Fertilizers- Biofertilizer — part -1: Rhizobial Specification and test method

1. Scope

This standard prescribes the requirements, method of sampling and tests for Rhizobial inoculants.

2. Normative reference

The following referenced documents are indispensable for the application of this Ethiopian standard. Only the edition of the documents (including any amendments) shall be applicable

ES ISO 14001: Environmental management systems - requirements with guidance for use

2. Terms and Definitions

For the purpose of this standard the following definitions shall apply:

2.1

biofertilizers

These are living organisms when seed-dressed, sown , applied to plant surface or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrient and/or growth stimulus to the target crop.

2.2.

inoculants

These are liquid or solid preparations containing one or more beneficial microorganisms in available state, intended for seed or soil application, designed to improve nutrient availability and help plant growth.

2.3.

carrier materials

These are materials used as support medium to maintain the viability of the inoculants until application.

2.4.

nodules

These are soft outgrowths on roots or stems particularly on leguminous plants resulting from the activity of some microorganisms which convert atmospheric nitrogen to ammonia .

2.5.

solubilisation

It is a process where non-available nutrients are dissolved to available form by microbial action.

2.6.

effectivness

It is the measurement of effectiveness of biofertilizer products in terms of plant vegetetive performance, dry mass or economic yield.

2.7.

lot

a single consignment of type of material belonging to the same batch of manufacture.

2.8.

batch

These are all inoculants that are prepared from a group of flasks/ fermentor .

2.10.

host

Plant that harbor any useful microorganisms.

2.11.

microbial group/species

These are microorganisms (bacteria, algae, fungi, protozoa) which aid soil health, and plant growth but excluding nematodes.

3.0. Requirements

3.1 General requirements

3.1. Rhizobial Biofertilizers shall:

3.1.1. contain competent, persistent and effective strain within its own agro-ecological conditions in minimum recommended population.

3.1.2. contain no more than the maximum allowed level of contamination.

3.1.3. have single or a combination of effective strains.

3.1.4. contain no pathogenic organisms that affect the biophysical environment.

3.1.5. contain carrier materials that are not harmful to the environment.

3.1.6. shall have the ability to fix atmospheric nitrogen.

3.1.7 should also have characteristics of mineral solubilisation/mobilization,ability to stimulate plant growth or Other desirable functions to inhance tolerance to pest damage and amelioration of the soil.

3.1.8. be effective and easy to apply and have adequate shelf life.

3.1.9. The manufacturing, use and even disposal of rhizobial shall be in conformance to ES ISO 14000 and stipulated in the Environmental Protection Act of the laws of Ethiopia.

3.2. Specific Quality requirements

Table 3.2 : Specific Quality requirement for Rhizobial biofertilzers

S.No	Parameters	Rhizobial	Test methods
3.2.1	Base	carrier based* or liquid based	A method in annex A or equivalent 3
3.2.2	Viable cell count 15 days after manufacturing	Minimum 10^7 colony forming unit /g on dry mass basis or 10^7/ml	
3.2.3	Viable cell count before 15 days of expiry	Minimum 10^6/g on dry mass basis or 106/ml	
3.2.4	Contamination level	No contamination at 10^{-5} dilution level	
3.2.5	pH	6.5-7.5	
3.2.6	Moisture % by weight (carrier based)	30-40% 3	
3.2.7	Efficiency character	Should show effective nodules on all the species listed on the packet	
3.2.8	Nitrogen fixation	Above 20mg per gm of glucose	
3.2.9	Shelf life	6 months after the date of manufacture	
3.2.10	Symbiotic effectiveness	> 50% of the dry mass as compared to the negative control or equal to the positive control at green house condition.	

***Type of carrier:** The carrier materials such as peat, lignite, peat soil, humus, wood charcoal or similar material favouring growth of organism.

4. Carrier materials specifications

4.1. Carrier materials for inoculants shall meet the following requirements:

4.1.1. The pH shall be readily adjusted to 6.5-7.0.

4.1.2. The carrier material shall have a high moisture-holding capacity (minimum of 20%)

4.1.3. The carrier shall be sterilized and free of lamp forming

4.1.4. The carrier shall be free of toxic materials.

4.1.5. The carrier shall contain sufficient quantity of carbon, greater than 20%

4.1.6. The sterilized carrier shall be free of any contamination

4.2. Particle size of carrier materials:

100 % of the Carrier material particle Shall pass through 150 micron sieve size

4.3. Packaging

4.3.1. Packaging material

Rhizobial Biofertilizers shall be packed in double packaging plastic materials as it allow gas exchange. The outer packing low density polyethylene (75-100 µm) material shall be colored but not black whereas the inner shall be transparent and high density polyethylene and steam steralizable. If sterilization is done with radiation, single low

density and opaque packaging plastic can be used. In principle the package system shall be easy to use and handle. Moreover, the containers, including packaging materials, used to package Rhizobial biofertilizers shall be made only of substances, which are safe and suitable for their intended uses. They shall not impart any toxic substance or undesirable odor to the desired microorganism.

4.3.2. Packaging size

The net weight of inoculants per packet shall be manufactured at 125g amount for seeds that meant for a seed lot enough to cover quarter of hectare.

5. Storage

Rhizobial Biofertilizer shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 15 °C to 30°C. 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. Transportation

Avoid direct sunlight, rain and temperatures shall not be less than 0°C and above 35°C during transportation.

7. Labeling

7.1. On the packaging bag, there shall be clear marks of:

I. Product name with its brand name

II. Batch number

III. Manufacturer/importer name and address.

VI. Appropriate host

V. Microbial population after 15 days of manufacturing

VI. Date of manufacture and expiry

VII. Net weight and the area meant for

VIII. Storage instructions worded as under: 'STORE IN COOLPLACE AWAY FROM DIRECT SUN AND HEAT'.

7.2. Direction for use shall be printed in a separate pamphlet and inserted in the packet.

8. Sampling

The method of drawing representative samples Rhizobial biofertilizers from different batches and the criteria for conformity shall be as follows: For ascertaining conformity of the material to the requirements of the specification, samples collected at random shall be tested from each lot (packets prepared on the same situation or date) separately.

Table 8.1. Number of packets to be selected for biofertilizers

No of packets in a lots of the same batch	Sample size*	Sample size**
Up to 1000	10	4
1001-5000 16		7
Above 5000	24	11

* = for requirements of microbial cell population, contamination level, pH and carrier size.

** = for requirements of effectiveness (nodulation and dry mass production)

ANNEX A

1. Carrier-based inoculants test

For carrier-based inoculants, the qualities to be checked are:

1. pH
2. Moisture content
3. Viable number microorganism
4. Plant infection method (MPN)

1.1. Determination of pH

Inoculants shall possess neutral pH conditions. Monitoring of the inoculants pH condition shall be made regularly in the following way; Make 20 g of the inoculant suspension in 50 ml of distilled water and shake on a rotary shaker for 2 h, filter on Whatman No. 1 filter paper or equivalent under vacuum using a funnel. Determine pH of the filtrate in a pH meter at 25 ^{o}C.

1.2 Moisture content

Weigh to the nearest mg about 10 gm of the prepared sample in a weighed clean, dry Petri Dish. Dry in an oven at 100 ^{o}C -105 ^{o}C to constant weight. Cool in a dessicator and weigh. Report percentage loss in weight as moisture content.

Calculation:

$$\text{Moisture percent by weight} = \frac{100(B-C)\#}{B-A}$$

A = Weight of the Petri Dish

B = Weight of the Petri dish plus material before drying

C = Weight of the Petri Dish plus material after drying

1.3 Viable number

The number of viable microorganism is counted by spread-plate method. Serially dilute one gram of the inoculant to obtain dilutions of the order of 10^{-5} to 10^{-6}. Plate 0.1 ml aliquots of the dilutions on YEMA plates and incubate at 28^{o}C ± 2°C for 3 to 7 days. The counts of viable microorganism in the inoculant shall be not less than 10^{7} Cfu/g of inoculants dry mass. Otherwise, the lot of inoculant to which this sample belongs shall be rejected.

1.4 Plant infection method (Efficiency character test for Rhizobial)

1.4.1 Principle: This is an indirect method of assessing plant infection on nodulation. It takes more time than spread plate method (because plants have to be grown). Most probable number (MPN) is usually done to compare the results with a spread plate method.

1.4.2 Assumptions:

- If a viable rhizobium is inoculated on its specific host, nodules will develop on those roots.
- Nodulation on that inoculated plant is a proof of the presence of infective rhizobia and when dissected, redish color should be observed.
- Absence of nodule is a proof of the absence of infective rhizobia.
- Uninoculated plants are used as control, with absence of nodule.

1.4.3 Estimation of MPN

Plants within any given pouch are considered as a growth unit. Nodulation is recorded + for 'nodulated growth unit' or – for absence of nodule. The actual number of nodules on each plant has no meaning on MPN count. If replications are in quadruplicated, the reading may be 4, 3,2,1 or 0 units. The highest dilution should show no nodulation. The estimated number rhizobium per g is calculated by the formula:

$$X = \frac{m \times d}{v}$$

Where

M = number from MPN Annex C

D = lowest dilution (first unit)

V= volume of aliquot inoculated.

Contaminants have some effect on counting .In the presence of contaminants; count of MPN will give lower results than plate counts.

1.5 Symbiotic effectiveness characterization

Testing the symbiotic effectiveness of inoculants is extremely crucial as it clearly assures the biomass and nitrogen accumulation capacity of the target microorganism on the intended crop with relative to control under green house.

$$\text{\% symbiotic effectiveness} = \frac{\text{Shoot dry weight of plants inoculated with test strain}}{\text{Shoot dry weight of plants supplied with Nitrogen}} \times 100$$

Annex C

Most Probable Numbers for use with 10 fold dilution and 5 tubes per dilution.

P_1	P_2	Most probable number for indicated values of P_3					
		0	1	2	3	4	5
0	0	-	0.018	0.036	0.054	0.072	0.090
0	1	0.018	0.036	0.055	0.073	0.091	0.11
0	2	0.037	0.055	0.074	0.092	0.11	0.13
0	3	0.056	0.074	0.093	0.11	0.13	0.15
0	4	0.075	0.094	0.11	0.13	0.15	0.17
0	5	0.094	0.11	0.13	0.15	0.17	0.19
1	0	0.020	0.040	0.060	0.080	0.10	0.12
1	1	0.040	0.061	0.081	0.10	0.12	0.14
1	2	0.061	0.082	0.10	0.12	0.16	0.17
1	3	0.089	0.10	0.13	0.16	0.17	0.19
1	4	0.11	0.13	0.15	0.17	0.19	0.22
1	5	0.13	0.15	0.17	0.19	0.22	0.24
2	0	0.046	0.068	0.091	0.12	0.14	0.16
2	1	0.068	0.092	0.12	0.14	0.17	0.19
2	2	0.093	0.12	0.14	0.17	0.19	0.22
2	3	0.12	0.14	0.17	0.20	0.22	0.25
2	4	0.15	0.17	0.20	0.23	0.25	0.28
2	5	0.17	0.20	0.23	0.26	0.29	0.32
3	0	0.078	0.11	0.13	0.16	0.20	0.23
3	1	0.11	0.14	0.17	0.20	0.23	0.27
3	2	0.14	0.17	0.20	0.24	0.27	0.31
3	3	0.17	0.21	0.24	0.28	0.31	0.35
3	4	0.21	0.24	0.28	0.32	0.36	0.40
3	5	0.25	0.29	0.32	0.37	0.41	0.45
4	0	0.13	0.17	0.21	0.25	0.30	0.36
4	1	0.17	0.21	0.26	0.31	0.36	0.42
4	2	0.22	0.26	0.32	0.38	0.44	0.50
4	3	0.27	0.33	0.39	0.45	0.52	0.59
4	4	0.34	0.40	0.47	0.54	0.62	0.69
4	5	0.41	0.48	0.56	0.64	0.72	0.81
5	0	0.23	0.31	0.43	0.58	0.76	0.95
5	1	0.33	0.46	0.64	0.84	1.1	1.3
5	2	0.49	0.70	0.95	1.2	1.5	1.8
5	3	0.79	1.1	1.4	1.8	2.1	2.5
5	4	1.3	1.7	2.2	2.8	3.5	4.3
5	5	2.4	3.5	5.4	9.2	16.0	--

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Ethiopian Standards Agency

Organization and Objectives

The Ethiopian Standards Agency (ESA) is the national standards body of Ethiopia established in 2010 based on regulation No. 193/2010.ESA is established due to the restructuring of Quality and Standards Authority of Ethiopia (QSAE) which was established in 1970.

ESA's objectives are:-

- ❖ Develop Ethiopian standards and establish a system that enable to check whether goods and services are incompliance with the required standards,
- ❖ Facilitate the country's technology transfer through the use of standards,
- ❖ Develop national standards for local products and services so as to make them competitive in the international market.

Ethiopian Standards

The Ethiopian Standards are developed by national technical committees which are composed of different stakeholders consisting of educational Institutions, research institutes, government or ganizations, certification, inspection, and testing organizations, regulatory bodies, consumer association etc. The requirements and/ or recommendations contained in Ethiopian Standards are consensus based that reflects the interest of the TC representatives and also of comments received from the public and other sources. Ethiopian Standards are approved by the National Standardization Council and are kept under continuous review after publication and updated regularly to take account of latest scientific and technological changes.
Orders For all Ethiopian Standards, International Standard and ASTM standards, including electronic versions, should be addressed to the Documentation and Publication Team at the Head office and Branch (Liaisons) offices. A catalogue of Ethiopian Standards is also a vailable freely and can be accessed in from our website.

International Involvement

ESA, representing Ethiopia, is a member of the International Organization for Standardization (ISO), and Codex Alimentarius Commission (CODEX). It also maintains close working relations with the in ternational Electro-technical Commission (IEC) and American Society for Testing and Materials (ASTM).It is a founding member of the African Regional Organization for standardization (ARSO).

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