KENYA STANDARD KS 2356: 2011

Bio fertilizer — Specification

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Kenya Organic Agriculture Network
Ministry of Agriculture
Mea Limited
Kenya Institute of Organic Farming
Osho chemicals
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Kenya Agricultural Research Institute
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In order to keep abreast of progress in industry, Kenya Standards shall be regularly reviewed. Suggestions for improvements to published standards, addressed to the Managing Director, Kenya Bureau of Standards, are welcome.

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Bio fertilizer — Specification

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Foreword

This Kenya standard has been prepared by the Technical Committees on Organic farming and organic products, and it is in accordance with the procedures of the Kenya Bureau of Standards.

Bio fertilizers are natural fertilizers which are natural inoculants of Bacteria, algae; fungi or a combination of all, and they enhance the availability of nutrients to plants
The use of bio fertilizers offers economic and ecological benefits by way of soil health and fertility.

This standard has been prepared to promote safe use of Bio fertilizers, promote fair trade practices and ensure safety of consumers.

During the preparation of this standard reference was made to the following documents:
EAS 456: 2007- East African Organic Products Standard
Microbial research centre (UON)Manual
The FNCA bio fertilizer 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
IPPC (ISPM -3Australian standard on Biofertilizers

Acknowledgement is hereby made for the assistance derived from the above sources

Bio fertilizer — Specification

1 Scope

This Kenya Standard specifies requirements for Bio fertilizers. It does not cover those derived from genetically modified organisms, manures and plant growth promoters. .

2. Definitions

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

2.1. Bio fertilizer

These are living organisms which 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, when applied to seed, plant surfaces, or soil.they may be propagated in different types of inorganic and organic carriers.

2.2. Inoculants

These are preparations containing beneficial micro-organisms in a viable state, intended for seed or soil application, designed to improve soil fertility and help plant growth by increasing the number of desired micro-organism in plant root zone

2.3 Bio fertilizer Carriers

These are substances which support and present organisms in a viable state

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2.3. Biological nitrogen Fixation

A process by which the atmospheric nitrogen gas is converted into forms that are easily absorbed by plants through biological processes.

2.4. Root nodules

Swellings or outgrowths on roots and stems particularly of leguminous plants resulting from the activity of some microorganisms which convert atmospheric nitrogen to .available nitrogen

2.5 plant growth promoters/enhancers/phytohormones

These are substances which stimulate the growth and development of plants

2.6. Symbiotic micro organisms

These are microorganisms that form a mutual relationship with plants in the nodules.

2.7. Mineralization

This is the biological breakdown of organic materials to release inorganic nutrients for plant uptake

2.8. Immobilization

A Process whereby inorganic nutrients are locked up in microbial cells thus rendering them unavailable to plants.

2.9. Solubilisation

A process, where bound nutrients are dissolved by microbial action .

2.10. Substrate

Any material that serves as source of nutrient for an organism.

2

3.0 Requirements

3.1 General Requirements

3.1.1. Bio fertilizer product shall contain effective strain in minimum recommended population.

3.1.3. Bio fertilizer product shall contain no more than the maximum allowed be contaminating microorganisms.

3.1.4. Bio fertilizer shall have single or a combination of effective strains.

3.1.5 Bio fertilizers shall contain no pathogenic organisms which could affect plants, animals and human beings.

3.1.5. For solid bio fertilizers the carrier used shall be of a nature that is not harmful to the environment **3.1.6** Bio fertilizer shall have at least one of the following effects:

a. Ability to fix nitrogen

b. Mineral solubilisation

c.

c)Bio fertilizer shall be able to stimulate plant growth.

d)Other desirable functions include: decreased pest incidence, fixing atmospheric nitrogen,mineral solubilisation,enhancing plant growth, stimulation of composting and amelioration of the soil.\

3.1.7

The use of micro organisms as bio fertilizers shall take into account of the persistence of the microorganism used as an inoculants vuis a vis the local conditions.

3.2. Specific Quality requirements

3.2.1. Bio fertilizer shall conform to the following requirements as set out in Table 1 to Table 4

Table 1: Specific Quality requirements of RHIZOBIA

Sl/no	parameter	Requirement
i)	Base	Carrier based or liquid based
ii)	Viable cell count(CFU)	Minimum 10^8 /g of carrier material or10^8/ ml of liquid
iii)	Contamination level	No contamination of 10^5 dilution
iv)	pH	6.5-7.5
v)	Particle size(carrier based)	All material shall pass through 0.15-0.212 mm IS sieve.
vii	Pathogenic microbes	Shall be nil
vi)	Moisture, % by weight, Max(carrier based)	30-40 %()
vii)	Efficiency character	Should show nodulation on all species(crops)listed on the packet
viii	Nodulation test	Should be positive

ix	Nitrogen fixation	Above 20 mg per g of glucose

Table 2: Specific Quality requirements of phosphate solubilising microorganisms

Sl/no	**parameter**	**Requirement**
i)	Base	Carrier(lignite/charcoal)
ii	Carrier size	➢ 100 micron
ii)	Viable cell count(CFU)	Minimum 10^7 /g of carrier material or10^7/ ml of liquid
iii)	Contamination level	No contamination of 10^4dilution
iv)	pH	6.5-7.5
v)	Particle size(carrier based)	All material shall pass through 0.15-0.212 mm IS sieve.more information
vii	Pathogenic microbes	Shall be nil
vi)	Moisture, % by weight, Max(carrier based)	35-40 %()
vii)	P -solubilisation	30-50%
viii	Shelf life	6 months
ix	*p- solubilisation zone*	I mm

Table 3: Specific Quality requirements of Azospirillum

Sl/no	**parameter**	**Requirement**
i)	Base	Carrier(lignite/charcoal)
ii	Carrier size	>100 micron
ii)	Viable cell count(CFU)	Minimum 10^7 /g of carrier material or10^7/ ml of liquid

iii)	Contamination level	No contamination of 10^4dilution
iv)	pH	7.0-8.0
v)	Particle size(carrier based)	All material shall pass through 0.15-0.212 mm IS sieve.more information
vii	Pathogenic microbes	Shall be nil
vi)	Moisture, % by weight, Max(carrier based)	35-40 %()
vii)	N-fixation	30-50%
viii	Shelf life	6 months
ix	*p- solubilisation zone*	I mm

Table 4: Specific Quality requirements of Azotobacter

Sl/no	**parameter**	**Requirement**
i)	Base	Carrier based
ii	Carrier size	Should pass through 106 micron IS sieve
ii)	Cell number	Minimum 10^7 /g of carrier material
iii)	Contamination level	No contamination of 10^5dilution
iv)	pH	6.5-7.5
v)	Particle size(carrier based)	All material shall pass through 0.15-0.212 mm IS sieve.more information
vii	Pathogenic microbes	Shall be nil
vi)	Moisture, % by weight, Max(carrier based)	35-40 %()
vii)	Nitrogen fixation	Not less than 10 mg/g of sucrose

viii	Shelf life	6 months
ix	*p- solubilisation zone*	I mm

4.0. Packaging

T

4.1.1 The Biofertilizer shall be packaged in materials that ensure the product integrity and quality.

4.1.2. The fill of the package shall comply with the weight and measures act CAP 513 of the laws of Kenya

4.1.3refer to kephis

5.0 Envronment

The disposal of condemned biofertilizer shall be done as stipulated in the Environmental Management and coordination act(EMCA)and CAP 324 0f the laws of Kenya.

6.0. Labelling

On the packaging bag, there must be clear marks of

i) product name,
ii) Brand name
iii) net weight, batch number, registration certificate number of the biofertilizer,

iv) manufacturer/importer name and physical address.

v) Microbial groups of microbes/species

vi)Crops to be applied

vii) Microbial density

viii) Date of manufacture

ix) Expiry date

x) Method of application

xi) Carrier composition (solid fertilizer

Xii) Storage conditions
xxiii) Compatibility
Xiv)) Declaration on GMO Status

ANNEX A (NORMATIVE)

PROCEDURES FOR QUALITY CONTROL OF BIO FERTILIZER

2.1 Rhizobium

Quality checks on Rhizobium biofertilizer can be divided into three parts:

1. Mother culture test
2. Broth test
3. Peat test

2.1.1. Mother culture test

Before producing Rhizobium bio fertilizer, the mother culture should be checked on the following:

1.1 Growth
1.2 Purity
1.3 Gram strain

Growth

By streaking a mother culture on yeast mannitol+Congo red agar (YMA) plates, checking the growth of rhizobia. Fast –Growing rhizobia colonies will appear in 3-5 days and slow-growing rhizobia will appear in 5-7 days.

Purity

Check purity by streaking culture on glucose peptone agar plate, and incubate for 24 hours at 30 °c.No growth or poor growth should be obtained on GPA. Good growth and colour changes can be expected from contaminants.

Gram stain

A lop of mother culture is checked by gram staining. Rhizobial cell is Gram-negative, retains safranin colour. Cells should appear red and not violet when observed under the microscope.

2.1.2. Broth test

The following qualities of the broth samples must be checked to make sure that the broths are in good condition.

2.1. PH

2.2. Staining
2.3. Optical density
2.4 Total count
2.5. Viable number

PH

Slow-growing rhizobia such as rhizobia for soybeans, mungbean and peanut produce a little basic compound. After incubation, the PH will increase.(example,PH before growing =6.0.after growing PH = 6.1-6.2) If broth PH decreases, it means some contaminants occur ;lower PH indicates presence of contaminants.

Staining (Gram stain or fushin stain)

Rhizobial cells are stained for observation of shape and size of the cells. Cells of rhizobia are rod-shaped, with one or two cells sticking together. They do not appear in long- chain. Long-chained cells are indicative of contaminants.

Gram- stained cells should appear red, not violet.Fuchsin staining is an easier and faster method. Rhizobial cells can be routinely checked using fuscshin stain.

Gram stain

Solutions		**Reactions and appearance of bacteria**	
		Gram-positive	**Gram-negative**
I.	Crystal violet(CV)	Cells stain violet	Cells stain violet
II.	Iodine solution	CV-I formed within cells; Cells remain violet	CV-I formed within cells; Cells remain violet

III	**Alcohol**	Cell walls dehydrated, Shrinkage of pores occurs, Permeability decreases, CV-I complex cannot pass out of cells, cells remain violet	Lipoid extracted from cell walls, porosity increases, CV-I is removed from cell
iv	**Safranin**	Cells not affected, remain violet	Cells take up this stain, become red.

2.3. Optical density

Broth culture with active rhizobial growth will become turbid in 3-4 days. Broth turbidity ,or optical density using spectrophotometer(at 540nm)will show readings of 0-1.0 o.d.The value of O.D correlates to number of cells. If Values are high then cells number are also high.

2.4. Total count

Total count includes viable cells and dead cells by using petroft-Hauser counter. At least 10 small squares all around the total area are counted, and not only in one large square.

Precautions:

1. cells have to be homogenous
2. clumping of cells(use non-ionic detergent
3. it gives total count only
4. Petroft, cover slip must be properly positioned to get uniform depth.

2.5. Viable count

The number of living cells is counted by spread plate or drop plate methods. Doing spread plate by making serial dilutions from 10^{-1} -10^{-6} or 10^{-7} (depend on concentration) then three replicates of 0.1millilitre of broth from 10^{-6} and 10^{-5} are spread over the YMA+CR plates. Plates are incubated in incubator (28-30^{o}c) or at room temperature for seven days. Colonies of rhizobial cells are round, opaque and have smooth margin. They are white and do not absorb red colour as well as other bacteria. Calculation of the number of rhizobia per ml;

$$\text{No of cells/ml} = \frac{\text{no. of colonies} \times \text{dilution factor}}{\text{Volume of inoculum}}$$

For example, no, of cells/ml=$\frac{32x10^6}{0.1}$=$32x10^7$

2.1.3. Peat test

For the peat inoculants, these are the qualities to be checked

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

2.1.3.1. PH

Maintain neutral PH for the innoculant. Since peat is acidic the PH is to be increased with $Caco_3$.Weigh 10 g of innoculant, pour 20 ml of distilled water, mix well with glass rod, incubate at least 30 minutes, and then measure with PH meter.

2.1.3.2 Moisture content

The optimum moisture content of peat-inoculant is between 40-50 %.At low moisture rhizobia will die rapidly. If moisture is high, inoculants may stick to the plastic bag and, thus, not good for rhizobial growth.

2.1.3.2.3 Viable number

The number of viable rhizobia is counted by spread-plate method as in the broth test. It is more difficult when analyzing non-sterile peat. Colonies may sometimes be contaminated by other bacteria. Good expertise is required to conduct this microbiological analysis.

2.1.3.2.4 Plant infection method

Principle: This is an indirect method of assessing plant infection on nodulation. It is widely used when peat is not sterile. It takes more time than spread plate method (because plants have to be grown).MPN is is usually done to compare the results with a spread plate method.

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.
- Absence of nodule is a proof of the absence of infective rhizobia.
- Uninoculated plants are used as control, with absence of nodule.

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:

$$X = \frac{mxd}{V}$$

Where

M=number from MPN table A.14.6 (Vincent 1970)

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.

2.2. Non symbiotic Nitrogen Fixer

In the laboratory, microbial growth may be represented by increase in cell mass, cell number or any cell constituent.

Utilization of nutrients or increase in metabolic products can also be related to the growth of the organism. Growth, therefore, can be determined by several techniques based on one of the following measurements:

a) Cell count directly by microscopy or by an electronic particle counter, or indirectly by colony count.

b) Cell mass, directly by weighing or measurement of cell nitrogen, or indirectly by turbidity.

c) Cell activity, indirectly by relating the degree of bio chemical activity to the size of the population.

The multiplication of *Azospirillum* is expected to have reached its maximal at 3-5 days after inoculation. Inoculants in autoclaved carriers are not expected to contain many inoculants. The recommended counting technique for BIO-N inoculants utilizing known volumes of serial dilutions is the drop plate method (Miles and Mistra).Plate dilutions are ranging from $10^{-4}$to 10^{-7}.If proper aseptic procedures are not fully observed, contaminants may be accidentally introduced during the introduction of the broth culture and during serial dilution and plating. Such contaminants will be detectable on these indicator media and their number should be reported together with their number of viable cells as a additional measure of the quality.

PROCEDURE

A. Dilution

1. Weigh 10g of BIO –N inoculants and inoculate it on 95ml of distilled water.

2. Shake vigorously and set aside

3. Make serial dilution of the 95ml inoculated with diluted BIO –N.To achieve this, set out 7 tubes each containing 9ml of the sterile diluents.

4. Use a fresh pipette tips for each dilution.

B. Plating

1. Use sterile enriched agar plates which are at least 3 days old or have dried at 37^0c for 2 hours.

2. Plate dilutions 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}

3. Allow the drops to dry by absorption into the agar; then invert and incubate at room temperature. Wrap the plates with sterile paper.

4. After 3-5 days of incubation with daily observations count the colonies of the respective organism of the BIO-N inoculants

5. Prefered counting range should be 10-30 colonies.

C. Computation

Example:

If the average number of colonies per drop is 30 at 10^{-5} dilution, the number of viable cells is:

$1/0.03 \times 30x10^{-5}=1000x10^{-5}$

$=1x10^{-2}$ ml

Enriched nutrient agar

S/N	Name of chemical	g/l
I)	Peptone	10
Ii)	NaCl	1
Iii)	Beef Extract	5
Iv)	Agar	18
v)	Distilled water	1,000ml

2.3. Mycorrhiza-the Arbuscular micorrhizal fungi, AMF

Quality control in the production of AMF inoculums is essential for product consistency, reliability and reproducibility. This is applied to the laboratory, preparation room, growth room, and storage room and green houses, taking care into the design to achieve the most efficient control in inoculums production.

2.3.1. Laboratory Quality control

1. Spores are extracted from selected batches of monospecific spore cultures in the preparation room.
2. The spores are transported in Petri dishes to the laboratory and placed in the refrigerator before examination.
3. The Petri dishes are examined under stereoscopic microscopes.
4. Description of the spores from each Petri dish is recorded.
5. Petri dishes are then cleaned and dried.

2.3.2. Preparation room quality control

i) This room has to be isolated from the green house and growth room and should not receive unsterilized soil or potting media samples

ii)Stored materials(cultures; sterilized growth media) are clearly labelled and placed in specific containers

iii) Floor should always be clean, avoiding sweeping which encourages distribution of dust

iv) Benches and other surfaces are cleaned with wet towels.

v) Containers are surface-sterilized with 10% sodium hypochlorite.

2.3.3. Growth room quality control

i) The growth room should be temperature controlled (22^0c) and air is exhausted to the outside (no recycling of stale air)

ii) Bench tops should be painted with anti microbial paint

iii) All surfaces should be sterilized periodically e.g. monthly.

iv)All samples are checked for contaminants and pathogens

v) Watering is done manually with great care to avoid cross-contamination

2.3.4. Storage room quality control

i) All samples stored are placed in plastic bags, with proper labelling and surface of bags should be wiped clean before storage.

ii) Floors and bench tops are wiped regularly, and dusting or sweeping should be avoided to prevent generation of dust.

2.4. Phosphate solubilizers

Phosphate solubilizers (PS) must contain phosphate solubilising bacteria or fungi. Commercially produced PS bio fertilizers must be certificated with guaranteed components such type of strains, microbial density, and biologicalactivity.

ANNEXXE B(NORMATIVE)

Properties of a good carrier material for seed inoculation

a)non-toxic to inoculants bacteria strain

b) Good moisture absorption capacity\

c) Easy tom process and free of lump-forming materials

d)easy to sterilize by autoclaving or gamma-irradiation.

e)Available in adequate amounts

f) Inexpensive

g) Good adhesion to seeds

h)Good pH buffering capacity

i)Non toxic to plant

PERMITTED CARRIERS FOR BIOFERTILIZERS

SL/NO	Carrier Material	Inoculum bacterium	characteristics
i	Sterilized	Rhizobium	_ seed

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	oxalic acid industrial waste		inoculation _Rhizobium multiplication in carrier in ambient temperature up to 90 days.	
ii	Alginate-perlite dry granule	Rhizobium	-soil inoculation _the inoculants can be stored in a dry state without losing much viability.	
iii	Composted sawdust	Bradyrhizobium,rhizobium and Azospirillum	_seed inoculation -good growth and survival of inoculants strains	
iv	Mineral soils	Rhizobium	-seed	

			inoculant	
v	coal	Rhizobium	_seed inoculant	
vi	Soybean oil or peanut oil added with lyophilized cells	Rhizobium	_seed inoculant _	
vii	Wheat bran,sugar cane baggers	Rhizobium/brady rhizobium and rock phosphate solubilising fungus *Aspergilus niger*	-soil inoculant	
viii	Nutrient supplemented pumice	Rhizobium	_seed inoculants	