Biofertilizers and Organic Fertilizers - Fertilizer (Control) Order, 1985

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National Centre of Organic Farming, Ghaziabad

Regional Centres :

Regional Centre of Organic Farming (HQ) Ghaziabad
Regional Centre of Organic Farming, Bengaluru
Regional Centre of Organic Farming, Bhubaneshwar
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Regional Centre of Organic Farming, Gandhinagar
Regional Centre of Organic Farming, Patna

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Biofertilizers and Organic Fertilizers Covered in Fertilizer (Control) Order, 1985

G.S.R. 758 (E). In exercise of the powers conferred by section 3 of the Essential Commodities Act, 1955 (10 of 1955), the Central Government hereby makes the following Order, namely

1. Short title and commencement

1. This Order may be called the Fertiliser (Control) Order, 1985.

2. It shall come into force on the date of its publication in the Official Gazette.

2. Definitions In this Order, unless the context otherwise requires:

(a) "Act" means the Essential Commodities Act, 1955 (10 of 1955).

(aa). Biofertiliser means the product containing carrier based (solid or liquid) living microorganisms which are agriculturally useful in terms of nitrogen fixation, phosphorus solubilisation or nutrient mobilization, to increase the productivity of the soil and/or crop.

b. "certificate of source" means a certificate given by a State Government, Commodity Board, manufacturer, + importer, pool handling agency or --as the case may be, wholesale dealer indicating therein the source from which fertiliser for purpose of sale is obtained.

c. "Commodity Board" means the Coffee Board constituted under section 4 of the Coffee Act, 1942 (7 of 1942) or the Rubber Board constituted under section 4 of the Rubber Act, 1947 (24 of 1947), or the Tea Board constituted under section 4 of the Tea Act, 1953 (29 of .1953), or as the case may be, the Cardamom Board constituted under section 4 of the Cardamom Act, 1965 (42 of 1965).

d. "compound or complex fertiliser" means a fertiliser containing two or more nutrients during the production of which chemical reaction takes place.

e. "controller" means the person appointed as Controller of Fertilisers by the Central Government and includes any other person empowered by the Central Government to exercise or perform all or any of the powers, or as the case may be, functions of the Controller under this Order.

(ee) “Customised fertiliser” means a granular multi nutrient carrier which contains Primary, Secondary and/or micro nutrient forms, both from inorganic and/or organic sources, manufactured through a systematic process of Fusion blend granulation, formulated on the basis of soil fertility data and include 100% water soluble speciality fertilizer as customized combination products.

f."Dealer" means a person carrying on the business of selling fertilisers whether wholesale or retail or industrial use* and includes a manufacturer, +Importer, and a pool handling agency carrying on such business and the agents of such person, manufacturer, +importer or pool handling agency.

g. Clause 'g' deleted vide S.O. 725 (E) dated 28.7.88.

h. "fer1i1iser" means any essential substance, either in straight or mixed form and derived from either inorganic, organic or mixed sources, that is used or intended to be used to provide essential plant nutrients or beneficial elements or both for the soil or for the crops or makes essential plant nutrients available to the plants either directly or by biological process or by both in the soil or plant as notified from time to time by Central Government and specified in the schedules appended to this order or as may be notified by the State Governments.

Explanation ;- For the purpose of Fertilizer-

(i) "the essential plant nutrients" include Primary Nutrients (Nitrogen, Phosphorus and Potassium), Secondary Nutrients (Calcium, Magnesium and Sulpher) and Micro Nutrients (Zinc, Manganese, Copper, Iron, Boron and Molybdenum);

(ii) "Beneficial element" means any element as notified by the Central Government from time to time.";

i. "Form" means a form appended to this Order.

j. "grade" means the nutrient element contents in the fertiliser expressed in percentage

k. "granulated mixture" means a mixture of fertilisers made by intimately mixing two or more fertilisers with or without inert material, and granulating them together, without involving any chemical reaction.

kk "importer" means a person who imports fertiliser in accordance with the Exportand Import Policy of the Central Government, as amended from time to time.

l. "inspector" means an Inspector of Fertilisers appointed under clause 27.

ll "industrial dealer" means a dealer who sells fertilisers for industrial purposes.

lll "industrial purposes" means the use of fertiliser for purposes other than fertilisation of soil and Increasing productivity of crops.

m. "manufacturer" means a person who produces fertlllsers or mixtures of fertilisers and the expression "manufacture" with its grammatical variations shall be construed accordingly.

ma. Marketer means such fertilizer companies who sell, offer for sale, carry on the business of selling of fertilizer manufactured by other fertilizer company.

n. "mixture of fertilisers" means a mixture of fertllisers made by physical mixing two or more fertilisers with or without inert material in physical or granular form and includes a mixture of NPK fertilisers, a mixture of micronutrient fertilisers and a mixture of NPK with micronutrient fertilisers.

nn Notified Authority "means an authority appointed under clause 26 A.

nna.Non-edible de-oiled cake fertilizer” means substance obtained as residue after oil extraction (by expeller and/or through solvent extraction) from crushed seeds of non-edible oilseeds (such as castor, neem) for use in soil as fertilizer.

o. "offer for sale" includes a reference to an intimation by a person of a proposal by him for the sale of any fertiliser, made by publication of a price list, by exposing the fertilizer for sale indicating the price, by furnishing of a quotation or otherwise howsoever.

oo. Organic fertilizer means substances made up of one or more unprocessed materials of a biological nature (plant/animal) and may include unprocessed mineral materials that have been altered through microbiological decomposition process.

p ‘physical mixture" means a mixture of fertilisers made by physically mixing two or more fertilisers with or without inert material necessary to make a required grade, without involving any chemical reaction.

(pp) “Provisional fertilizer” means fertilizer specified under clause 20 A’.

q. "prescribed standard" means:-

i. in relation to a fertiliser included in column 1 of Part A of Schedule-I, the standard set out in the corresponding entry in column 2, subject to the limits of permissible variation as specified in Part B of that Schedule; and

ii. in relation to a mixture of fertilisers, the standard set out in respect of that mixture under sub-clause (1) of clause 13 by the Central Government, subject to the limits of permissible variation as 4 specified in Part B of Schedule-l

iii. in relation to mixture of fertilisers, standard set out in respect of that mixture under sub-clause (2) of clause 13 by the State Government, subject to limits of permissible variation as specified in Part B of Schedule-l.

iv. in relation to a Biofertiliser included in column 1 of Part A of Schedule-III, the standard set out in the corresponding entry in column 2, subject to the limits of permissible variation as specified in Part B of that Schedule;

v. in relation to aOrganic fertiliser included in column 1 of Part A of Schedule-IV, the standard set out in the corresponding entry in column 2, subject to the limits of permissible variation as specified in Part B of that Schedule.

vi. In relation to a Non-edible, De-oiled cake fertilizer specified in column (2) of part A of schedule V, the standard set out in the corresponding entry in column (2) of the said part, subject to the limits of permissible variation as specified in part B of that schedule”.

vii. Prescribed standard means in relation to Customized Fertilizers standards set out in respect of Customized Fertilizers under clause 20B by the Central Government, subject to limits of permissible variation as specified in part B of Schedule-I

r. "pool handling agency" means an agency entrusted by the Central Government with functions relating to handling and distribution of imported fertilisers.

s. "registering authority" means a registering authority appointed under clause 26 in respect of mixture of fertilizers and special mixture of fertilizers

t. "retail dealer" means a dealer who sells fertilisers to farmers or plantations for agricultural use such as for fertilisation of soil and increasing productivity of crops.

u. "Schedule" means a Schedule appended to this Order.

v. "special mixture of fertilisers" means any mixture of fertilisers prepared for experimental purposes in pursuance of a requisition made by any person (including a person engaged in the cultivation of tea, coffee or rubber) for sale to that person in such quantity and within such period as may be specified in such requisition; and.

w. "wholesale dealer" means a dealer who sells fertilisers otherwise than in retail-for agricultural use such as for fertilisation of soil and increasing productivity of crops.

II. PRICE CONTROL

3. Fixation of prices of fertilisers

1. The Central Government may, with a view to regulating equitable distribution of fertilisers and making fertilisers available at fair prices, by notification in the Official Gazette, fix the maximum prices or rates at which any fertiliser may be sold by a dealer, manufacturer, +importer or a pool handling agency.

2. The Central Government may having regard to the local conditions of any area, the period of storage of fertilisers and other relevant circumstances, fix different prices or rates for fertilisers having different periods of storage or for different areas or for different classes of consumers.

3. No dealer, manufacturer +importer or pool handling agency shall sell or offer for sale any fertiliser at a price exceeding the maximum price or rate fixed under this clause.

4. Display of stock position and price list of fertilisers

Every dealer, who makes or offers to make a retail sale of any fertilisers, shall prominently display in his place of business:-

a. the quantities of opening stock of different fertilisers held by him on each day;
   Explanation -The actual stocks at any point of time during the day may be different from that of the displayed opening stocks to the extent of sale and receipt of such fertilisers upto the time of inspection during that day

b. a list of prices or rates of such fertilisers fixed under clause 3 and for the time being in force.

5. Issue of cash/credit memorandum

Every dealer shall issue a cash or credit memorandum to a purchaser of a fertiliser in Form M

III. CONTROL ON DISTRIBUTION OF FERTILISERS BY MANUFACTURER/ IMPORTER

6. Allocation of fertilisers to various States

The Central Government may, with a view to securing equitable distribution and availability of fertilisers to the farmers in time, by notification in the Official Gazette, direct any manufacturer/importer to sell the fertilisers produced by him in

such quantities and In such State or States and within such period as may be specified in the said notification.

IV. AUTHORISATION OR REGISTRATION OF DEALERS

7. Registration of Industrial dealers and authorization of other dealers

No person shall sell, offer for sale or carry on the business of selling of fertilizer at any place as wholesale dealer or retail dealer except under and in accordance with clause8:

Provided that a State Government may, if it considers it necessary or expedient, by notification in the Official Gazette, exempt from the provisions of this clause any person selling fertilizer to farmers in such areas and subject to such conditions as may be specified in that notification.

8. Application for intimation or registration

1. Every person intending to sell or offer for sale or carrying on the business of selling of fertilizer as Industrial Dealer shall obtain a certificate of registration from the controller by making an application in Form A together with the fee prescribed under clause 36 and a Certificate of source in Form O.

2. Every person including a manufacturer, an importer, a pool handling agency, wholesaler and a retail dealer intending to sell or offer for sale or carrying on the business of selling of fertilizer shall make a Memorandum of Intimation to the Notified Authority, in Form A1 duly filled in, in duplicate, together with the fee prescribed under clause 36 and certificate of source in Form O.

3. On receipt of a Memorandum of Intimation, complete in all respects, the Notified Authority shall issue an acknowledgement of receipt in Form A2 and it shall be deemed to be an authorization letter granted and the concerned person as authorised dealer for the purposes of this Order.

Provided that a certificate of registration granted before the commencement of the Fertiliser (Control) Amendment Order, 2003, shall be deemed to be an authorization letter granted under the provisions of this Order:

Provided further that where the applicant is a State Government, a manufacturer or an importer or a pool-handling agency, it shall not be necessary for it or him to submit Form O.

Provided also that a separate Memorandum of Intimation shall be submitted by an applicant for whole sale business or retail dealership, as the case may be:

Provided also that where fertilizers are obtained for sale from different sources,a certificate of source from each such source shall be furnished in Form O."

Provided also that where the manufacturer of organic fertilizer is a State Government or municipality, it shall not be necessary for it to obtain the authorization letter;

Provided also that where the manufacturer of vermi-compost, other than a State Government or municipality, has annual production capacity less than 50 metric tonnes, it shall not be necessary for him to obtain the authorization letter.

4. No authorization letter shall be granted to any applicant for retail dealership, unless the applicant possess the certificate course of fifteen days from any State Agriculture University or Krishi Vigyan Kendras or National Institute of agricultural extension management (MANAGE) or National Institute of Rural Development and Panchayat Raj (NIDPR) or Fertilizer Association of India or any other approved Governemnt Institute:

Provided that a person in possession of Bachelor of Science in Agriculture or Chemistry or Diploma in agriculture science from a recognized industry or institute or equivalent course having one of the subject on fertilizer or agri inputs, as notified by the State government shall not be required to possess separate certificate course:

Provided that a dealer who has been granted authorization letter before commencement of the Fertilizer (inorganic, organic or mixed) (control) Fourth Amendment Order, 2018 shall not be required to possess the qualification at the time of renewal of their authorization letter.

Provided also that the said qualification shall not be applicable for renewal of the authorization letter of the registered agricultural cooperative society and state marketing federations subject to condition that such Society or Federation shall engage a person who possesses the qualification under this clause.

9. Grant or refusal of certificate of registration

The Controller, shall grant a certificate of registration in Form 'B' within thirty days of the receipt of application to any person who applies for it under clause 8;

Provided that no certificate of registration shall be granted to a person: -

a. if his previous certificate of registration is under suspension; or

b. if his previous certificate of registration has been cancelled within a period of one year immediately preceding the date of application; or

c. if he has been convicted of an offence under the Act, or any Order made there under within three years immediately preceding the date of making the application

d. if he fails to enclose with the application a certificate of source ; or

e. if the application is incomplete in any respect; or

f. if he makes an application for obtaining the certificate of registration for industrial dealer and, excepting if he is a manufacturer ,+ importer or pool handling agency, holds [an authorization letter] for wholesale dealer or retail dealer or both, and as the case may be, the vice-versa.

10. Period of validity of certificate of registration and letter of authorization Every certificate of registration granted under clause 9 and every authorization letter issued under clause 8 shall, unless renewed, suspended or cancelled, be valid for a period of three years from the date of its issue.

Notwithstanding anything contained in the said clause, the letter of authorization granted to the manufacture of city compost issued under clause 8, unless suspended or cancelled is valid in perpetuity.

11. Renewal of certificates of registration and authorization letters

1. Every holder of a certificate of registration granted under clause 9 or authorization letter granted or deemed to have been granted under clause 8, desiring to renew such certificate or authorization letter shall, before the date of expiry of such certificate of registration or authorization letter, as the case may be, make an application for renewal to the Controller, in Form C, or to the Notified Authority in Form A1, respectively, in duplicate, 8 together with the fee prescribed under clause 36 for such renewal and a certificate of source as required under clause 8.

2. On receipt of an application under sub-clause (1), together with such fee and certificate of source, the controller may renew the certificate of registration or the Notified Authority, as the case may be shall issue acknowledgement receipt of renewal in form A 2.

Provided that a certificate of registration shall not be renewed if the holder of the same did not sell any fertiliser during the period of one year immediately preceding the date of expiry of the period of validity.

3. If any application for renewal is not made before the expiry of the period of validity of the certificate of registration or, as the case may be, the authorization letter but is made within one month from the date of such expiry, the certificate of registration or, as the case may be, the authorization letter shall be dealt as provided in sub-clause (2) on payment of such additional fee as may be prescribed under clause 36 in addition to the fee for renewal.

4. Where the application for renewal of certificate of registration is made within the time specified in sub- clause (1) or sub-clause (3), the applicant shall be deemed to have held a valid certificate of registration until such date as the controller passes orders on the application for renewal.

5. If an application for renewal of a certificate of registration or authorization letter is not made within one month from the date of expiry of their period of validity ,the same shall be deemed to have lapsed on the date on which its validity expired and any business carried on after that date shall be deemed to have been carried on in contravention of clause 7."

V. MANUFACTURE OF MIXTURES OF FERTlLIZERS,

12. Restriction on preparation of mixtures of fertilizer No person shall carry on the business of preparing any mixture of fertilisers. or special mixture of fertilizers, Bio-fertilizers or Organic fertilisers except under and in accordance with the terms and conditions of a certificate of manufacture granted to him under clauses 15 or 16.

13. Standards of mixtures of Fertilisers

1. Subject to the other provisions of the order

(a) no person shall manufacture any mixture of fertilisers whether of solid or liquid fertilizers specified in Part a of schedule I unless such mixture conforms to the standards set out in the notification to be issued by the Central Government in the Official Gazette;

(b) no person shall manufacture any biofertiliser unlessl such biofertiliser conforms to the standards set out in the part A of Schedule – III.

(c) no person shall manufacture any Organic fertilizer unless such organic fertilizer conforms to the standards set out in the part A of Schedule IV.

2. Subject to the other provisions of this order, no person shall manufacture any "mixture of fertilisers unless such mixture conforms to the standards set out in the notification to be issued by the State Government in the Official Gazette;

Explanation- For the purposes of this sub-clause, mixture of fertilizers shall not include liquid fertilizers and 100% water soluble fertilizers, containing N,P,K.

3. [omitted]

4. No Certificate of manufacture shall be granted in respect of any fertiliser which does not conform to the standards set out in the notification referred in sub- clause (1) or (2);

5. Nothing in this clause shall apply to special mixtures of fertilisers

14. Application for certificate of manufacture of mixtures of fertillsers

1. Every person desiring to obtain a certificate of manufacture for preparation of any mixture of fertilisers or special mixture of fertilisers shall possess such mixture, *and possess the minimum laboratory facility as specified in clause 21A of this Order.

2. An applicant for a certificate of manufacture for preparation of mixture of fertilisers or special mixture of fertilisers shall make an application to the registering authority

a. if he is an applicant for a certificate of manufacture for any mixture of fertilisers in Form D, in duplicate, together with the fee prescribed there for under clause 36; or,

b. if he is an applicant for a certificate of manufacture for any special mixture, in Form D, in duplicate, together with the fee prescribed there for under the said clause 36 and an attested copy of the requisition of the purchaser.

15. Grant or refusal of certificate of manufacture for preparation of mixtures of fertilizers, Biofertilisers or Organic fertilizer.

1. On receipt of an application under clause 14, the registering authority shall, by order in writing, either grant or refuse to grant the certificate of manufacture in respect of any mixture of fertilizer, Biofertiliser, Organic fertiliser or special mixture of fertilizer and shall, within forty-five days from the date of receipt of the application, furnish to the applicant a copy of the order so passed;

2. Where an application for a certificate of manufacture for mixture of fertilizers, Biofertiliser, Organic fertiliser is not refused under sub-clause (1), the registering authority shall grant a certificate of manufacture in Form F and where an application for a certificate of manufacture for a special mixture is not refused under that sub-clause, [such authority shall within forty five dates from the date of receipt of the application,]grant a certificate of manufacture to the applicant in Form G

16. Conditions for grant of certificate of manufacture in respect of special mixture of fertilisers and period of validity of such certificate

1. No certificate of manufacture in respect of any special mixture of fertilisers shall be granted to an applicant unless he holds a valid certificate of manufacture under this Order for any mixture of fertilisers.

2. Every certificate of manufacture granted in respect of any special mixture of fertilisers shall be valid for a period of [sixmonths] from the date of its issue;

Provided that the registering authority may, if it is satisfied that it is necessary so to do, extend the said period to such further period or periods as it may deem fit, so however, that the total period or periods so extended shall not exceed [twelve months]

17. Period of validity of a certificate of manufacture for preparation of mixtures of fertilizers, Biofertilisers or Organic fertilizer.

Every certificate of manufacture granted under clause 15 for preparation of a mixture of fertilizers, Biofertiliser or Organic fertilizers shall, unless suspended or cancelled, be valid for a period of three years from the date of issue.

18. Renewal of certificate of manufacture for preparation of mixtures of fertilizers, Biofertiliser or Organic fertiliser

1. Every holder of a certificate of manufacture for preparation of a mixture of fertilizers, Biofertiliser, Organic fertiliser desiring to renew the certificate, shall, before the date of expiry of the said certificate of manufacture make an application to the registering authority in Form D in duplicate, together with the fee prescribed for this purpose under clause 36.

2. On receipt of an application for renewal as provided in sub-clause (1), and keeping in view the performance of the applicant and other relevant circumstances, the registering authority may, if he so decides, renew the [certificate of manufacture by endorsement on Form F and in case the certificate of 11 registration is not renewed, the registering authority shall record in writing his reasons for not renewing the certificate of manufacture.

3. If an application for renewal is not made before the expiry of the certificate of manufacture but is made within one month from the date of expiry of the [certificate of manufacture, the certificate of manufacture] may be renewed on payment of such additional fee as may be prescribed by the State Government for this purpose.

4. Where the application for renewal is made within the time specified in subclause (1) or sub-clause (3), the applicant shall be deemed to have held a valid [certificate of manufacture] until such date as the registering authority passes order on the application for renewal.

5. f an application for renewal of a certificate of manufacture is not made within the period stipulated under sub-clause (1) or, as the case may be, under subclause (3), the certificate of manufacture shall be deemed to have expired immediately on the expiry of its validity period, and any business carried on after that date shall be deemed to have been carried on in contravention of clause 12.

VI. RESTRICTIONS ON MANUFACTURE/ IMPORT, SALE, ETC. OF FERTlLISER

19. Restriction on manufacture/import, sale and distribution of fertilisers

No person shall himself or by any other person on his behalf:-

a. manufacture/import for sale, sell, offer for sale, stock or exhibit for sale or distribute any fertlliser which Is not of prescribed standard;

b. manufacture/Import for sale, sell, offer for sale, stock or exhibit for sale, or distribute any mixture of fertl11sers, which is not of prescribed standard (subject to such limits of permissible variation as may be specified from time to time by the Central Government) or special mixture of fertilisers which does not conform to the particulars specified In the certificate of manufacture granted to him under this Order in respect of such special mixture.

c. sell, offer for sale, stock or exhibit for sale or distribute:-
i. any fertiliser the container whereof is not packed and marked in the manner laid down In this Order

ii. any fertiliser which is an [imitation of or] a substitute for another fertiliser under the name of which It Is sold;

iii. any fertiliser which Is adulterated;

Explanation:- A fertiliser shall be deemed to be adulterated, If It contains any substance the addition of which is likely to eliminate or decrease Its nutrient contents or make the fertiliser not conforming to the prescribed standard.

iv. any fertiliser the label or container whereof bears the name of any individual firm or company purporting to be manufacturer/Importer of the fertiliser, which individual, firm or company Is fictitious or does not exsist.

v. any fertiliser, the label or container whereof or anything accompanying therewith bears any statement which makes a false claim for the fertiliser of which s false or misleading in any material particular.

vi. any substance as a fertiliser which substance is not, in fact, a fertiliser; or

vii any fertilizer without exhibiting the minimum guaranteed percentage by weight of plant nutrient.

Provided that specifications of city compost in Schedule IV shall, in case of municipalities, be applicable only when it is traded in packaged form for use in agriculture:

Provided further that the specification of vermin-compost in Schedule IV shall be applicable only in such cases where it is sold in packaged form and for agricultural purposes.

viii. "Provided also that the specifications of non-edible de-oiled cake fertilizer in Schedule V shall be applicable only in such case where it is sold in packaged form for agricultural purposes."

20. Specifications In respect of imported fertilisers

Notwithstanding anything contained in this Order, the Central Government may by an order, published in the Official Gazette, fix separate specifications in respect of imported fertilisers.

20 A. Specification in respect of provisional fertilizer

Notwithstanding anything contained in this Order, the Central Government may, by order published in the Official Gazette, notify specifications, valid for a period not exceeding three years, in respect of fertilizers to be manufactured by any manufacturing unit for conducting commercial trials.

20 B.- Specifications in respect of customized fertilizers.–

i. Notwithstanding anything contained in this Order, the Central Government may by order published in the Official Gazette, notify specification, valid for a period not exceeding three years in respect of customized fertiliser

ii. No person shall manufacture any grade of Customised fertilizer unless such customized fertilizer conforms to the standards set out in the notification to

be issued by the central government in the official gazette under sub-clause (i)

Provided that the gardes of customized fertilizer which the company will manufacture must be based on the soil fertility data maintained by the Ministry of Agriculture and Farmers Welfare or State government:

Provided further that in case the data for a district for which the company intending to formulate the grade is not available or still under process by the state government then the company shall use the scientific data obtained from soil testing result generated by testing in their own laboratories.

iii. No person except with the prior permission of the controller shall manufacture any particular grade of customized fertilizer formulated as per the general specification noted under sub-clause (i)

iv. Every person desirous of obtaining a specific product approval of any particular grade of customized fertilizer shall make an application in form Q in duplicate to the Controller of Fertilizer Government of India.

v. On receipt of application under clause (ii) the Controller shall by order in writing either grant or refuse to grant the permission, in respect of manufacturing of any particular grade of customized fertilizer and shall within three months from the date of receipt of application shall furnish a copy of order so passed to the applicant.

Provided that on completion of three years or earlier, manufacturing company of customized fertilizer shall again submit an application for approval for manufacturing of the said grade:

Provided further that the permission for manufacture and sale of Customized fertilizer shall be granted to only such fertilizer companies whose annual production of fertilizers other than CFs is 5.00 lakh metric tonne:

Provided also that such manufacturing companies, having annual production of 5 LMT of fertilizer other than CFs, can set up manufacturing units of CF either on their own or through subsidiaries or joint venture through a minimum stake of 51% in such joint ventures.

21. Manufacturers/Importers pool handling agencies to comply with certain requirements in regard to packing and marking, etc.

Every manufacturer/importer and pool handling agency shall, in regard to packing and marking of containers of fertilizers, Biofertiliser or Organic fertiliser comply with the following requirements, namely:-

a. Every container in which any fertilizer is packed shall conspicuously be superscribed with the word “FERTILISER” and shall bear only such particulars and unless otherwise required under any law nothing else, as may from time to time, be specified by the Controller in this behalf,

   Provided that in case of containers the gross weight of which is 5 kg or less, no such printing of superscription and other particular shall be

necessary if such super superscription and other particulars are printed on a separate label which is securely affixed to such container

(aa) Every container in which any Biofertiliser or Organic fertilizer is packed shall conspicuously be superscribed with the word "BIO-FERTILISER/ ORGANIC FERTILISER OR NON-EDIBLE DE-OILED CAKE FERTILZER as the case may be" and shall bear only such particulars and unless otherwise required under any law nothing else, as may from time to time, be specified by the Controller in this behalf,

. (b) Every container shall be so packed and sealed that the contents thereof cannot be tampered with without breaking the seal;

Provided that where fertilizer manufactured in India are packed in bags stitched on hand, such bags shall bear lead seals, so that the contents thereof cannot be tampered with without breaking the seals;

Provided further that lead sealing shall not be necessary:-

(i) if such bags are machine stitched in such a manner that contents thereof cannot be tampered with without a visible break in the stitching; and

(ii) in the case of fertilizers imported from abroad and packed a in bags stitched in hand, in such a manner that the contents thereof cannot be tampered with without visible break in the stitching. P

Provided also that in case fertilizer bags are in cut, torn or damaged condition during transportation or mishandling during loading or unloading operation, the manufacturer of such fertilizer may, under intimation to the State Government and the Central Government, repack he fertilizer in new bags or restandardise the quantity in terms of declared weight.

c.Every fertiliser bag in which any fertiliser is packed for sale shall be of such weight and size as may be specified by the Central Government from time to time in this behalf

21 A. Manufacturers to comply with certain requirements for laboratory facilities:-

Every manufacturer shall, in order to ensure quality of their product, possess minimum laboratory facility, as may be specified from time to time by the Controller.

22. Bulk sale of fertillsers

Notwithstanding anything contained In this Order:-

a. a retail dealer may retain at any time one bag or container of each variety of fertiliser in an open and unsealed condition for the purpose of sale;

b. a manufacturer/importermay sell the fertillser manufactured/imported by him in bulk to a manufacturer of mixture of fertilisers, compound / complex fertilisers or special mixture of fertilisers; and

b. the Central Government may by notification published in the Official Gazette in this behalf authorise a manufacturer/importer to sell any fertiliser manufactured/ imported by him In bulk also direct to farmers for such period as may be specified in that notification:

Provided that a certificate indicating the minimum guaranteed percentage of plant nutrients is issued by the manufacturer/importer to each farmer at the time of such sale.

23. Disposal of non-standard fertilisers

1. Notwithstanding anything contained In this Order, a person may sell, offer for sale, stock or exhibit for sale or distribute, [any fertillser except any fertillser imported by the Central Government] which, not being an adulterated fertiliser, does not conform to the prescribed standard (hereinafter in this Order referred to as non-standard fertiliser) subject to the conditions that:-

a. the container of such non-standard fertilizer is conspicuously superscribed in red colour with the words "non-standard" and also with the sign "X"; and

b. an application forthe disposal of non-standard fertilisers in Form H is submitted to the [Notified authority] to grant a certificate of authorisation for sale of such fertilisers and a certificate of authorisation with regard 15 to their disposal and price is obtained in Form I.

c. such non-standard fertiliser shall be sold only to the manufacturers of mixtures of fertilisers or special mixtures of fertilisers or research farms of Government or Universities or such bodies.

2. The price per unit of the non-standard fertiliser shall be fixed by the [notified authority] after satisfying itself that the sample taken is a representative one, and after considering the nutrient contents in the sample determined on the basis of a chemical analysis of the nonstandard fertilizer.

3. The Central Government may, by notification in the official Gazette and subject to the conditions, if any, laid down in that notification, and subject to guidelines issued in this regard by the Central Government exempt such pool handling agencies, as it deems fit, from complying with conditions laid down in paragraphs (a) and (b) of the sub-clause (1)

4. Where any fertiliser imported by the Central Government is found to be of non-standard and the Central Government decides that the fertilizer cannot be permitted for direct use in agriculture, it may permit the use of

such fertiliser by manufacturers of complex fertilisers, mixture of fertilisers or special mixture of fertilisers to be sold at such price as may be fixed by the Central Government.

5. If a manufacture or importer detects or as reasonable doubt about the standard of the fertilizer manufactured or imported by him, and dispatched for sale as deteriorated in quality during transit due to natural calamity and is not of the prescribed standards, he may, within fifteen days from the date of dispatch from factory or port, apply with detailed justifications to the Central Government for obtaining permission for reprocessing the same in a factory to meet the prescribed standards and the Central Government may, after considering the facts, permit the re-processing of such fertilizer on the terms and conditions as may be notified by the Central Government in this behalf.

Provided that no such application for permission to reprocess the fertilizer by the manufacturer or importer shall be accepted by the Central Government after the expiry of the said period of fifteen days.

24. Manufacturers/Pool handling agencies to appoint officers responsible with compliance of the Order

Every manufacturing organization, importer and pool handling agency shall appoint in that organization and in consultation with the Central Government, 16 an officer, who shall be responsible for compliance with the provisions of this Order .

25. Restriction on sale/use of fertilisers

1. No person shall, except with the prior permission of the Central Government and subject to such terms and conditions as may be imposed by such Government, sell or use fertiliser, for purposes other than fertilisation of soils and increasing productivity of crops.

Provided that the price of fertilisers permitted for sale for industrial use shall be no profit no loss price, excluding all subsidies at the production, import, handling or on sale for agricultural consumers;

Provided further that wherever customs or excise duties are chargeable, these may be added to the price so fixed.

2. Notwithstanding anything contained in sub-clause (1), no prior permission for use of fertiliser for industrial purposes shall be necessary when the fertiliser for such purposes is purchased from the Industrial dealer possessing a valid certificate of registration granted under clause 9.

3. Any person possessing a valid certificate of registration for Industrial dealer, unless such person is a State Government, a manufacturer/importer or a pool handling agency, shall not carry on the business of selling fertilisers foragricultural purposes, including a

wholesale dealer or a retail dealer. However, in case of a State Government, a manufacturer or a importer or a pool handling agency possessing a valid certificate of registration for sale of fertiliser for industrial use, and also for sale of fertiliser for agricultural use, whether in wholesale or retail or both, shall not carryon the business of selling fertilisers both for Industrial use and agricultural use In the same premises.

VII. ENFORCEMENT AUTHORITIES

26. Appointment of registering authority

The State Government may, by notification in the Official Gazette, appoint such number of persons, as it thinks necessary, to be registering authorities for the purpose of this Order [$]for industrial dealers, and may, in any such notification define the limits of local 17 area within which each such registering authority shall exercise his jurisdiction.

26A. Notified Authority- The State Government may, by notification in the Official Gazette, appoint such number of persons, as it thinks necessary, to be Notified Authorities for the purpose of this Order and define the local limits within which each such Notified Authority shall exercise his jurisdiction.

27. Appointment of inspectors The State Government, or the Central Government may, by notification in the Official Gazette appoint such number of persons, as it thinks necessary, to be inspectors of fertilisers for the purpose of this Order, and may, in any such notification, define the limits of local area within which each such inspector shall exercise his jurisdictions.

27A. Qualifications for appointment of fertiliser Inspectors

No person shall be eligible for appointment as Fertiliser Inspector under this Order unless he possesses the following qualifications, namely:-

1. Graduate In agriculture or science with chemistry as one of the subjects, froma recognlsed university; and

2. Training or experience in the quality control of fertilisers and working in the State or Central Government Department of Agriculture.

27AA. Regular training of fertilizer Inspectors

Every Fertilizer inspector shall undergo training after every three years in the Central Fertilizer Ouality Control and Training Institute or any Regional Fertilizer Quality Control laboratory at Mumbai, Kalyani or Chennai.

27B. Qualifications for appointment of fertiliser Inspectors for Biofertiliser, Organic Fertiliser and Non edible de-oiled cake fertilizer.

No person shall be eligible for appointment as inspector of biofertiliser and Organic fertilizer under this Order unless he may possess the following qualifications, namely:

(1) Graduate in agriculture or science with chemistry/microbiology as one of the subject; and

(2) Training or experience in the field of quality control of biofertilisers/organic fertilizers/non edible de-oiled cake fertilizer.

28. Powers of Inspectors

1. An inspector may, with a view to securing compliance with this Order:-

a. require any manufacturer, +importer, pool handling agency, wholesale dealer or retail dealer to give any information in his possession with respect to the manufacture, storage and disposal of any fertilizer manufactured or, in any manner handled by him

b.draw samples of any fertiliser in accordance with the procedure of drawal of samples laid down in Schedule II.

Provided that the inspector shall prepare the sampling details in duplicate In Form J, and hand over 18 one copy of the same to the dealer or his representative from whom the sample has been drawn;

(ba) draw samples of any biofertilisers in accordance with the procedure of drawl of samples laid down in schedule III.

Provided that the inspector shall prepare the sampling details in duplicate in form J and hand over one copy of the same to the dealer or his representative from whom the sample has been drawn;

(bb) draw samples of any organic fertilisers in accordance with the procedure of drawl of samples laid down in schedule IV.

Provided that the inspector shall prepare the sampling details in duplicate in form J and hand over one copy of the same to the dealer or his representative from whom the sample has been drawn;

(bc)draw samples of any non- edible de -oiled cake in accordance with the procedure of drawl of samples laid down in schedule V

Provided that the inspector shall prepare the sampling details in duplicate in form J and hand over one copy of the same to the dealer or his representative from whom the sample has been drawn;

c. enter upon and search any premises where any fertiliser is manufactured/ Imported or stored or exhibited for sale, if he has reason to believe that any fertiliser has been or is being manufactured/imported, sold, offered for sale, stored, exhibited for sale or distributed contrary to the provisions of this Order;

d. seize or detain any fertiliser in respect of which he has reason to believe that a contravention of this Order has been or is being or is [attempted] to be committed;

e. seize any books of accounts or documents relating to manufacture, storage or sale of fertilisers, etc. in respect of which he has reason to believe that any contravention of this Order has been or is being or is about to be committed;

Provided that the Inspector shall give a receipt for such fertilisers or books of accounts or documents so seized to the person from whom the same have been seized;

Provided further that the books of accounts or documents so seized shall be returned to the person from whom they were seized after copies thereof or extracts thereform as certified by such person, have been taken.

2. Subject to the proviso to paragraphs (d) and (e) of sub-clause (1), the provisions of the Code of Criminal Procedure, 1973 (2 of 1974) relating to search and seizure shall, so far as may be, apply to searches and seizures under this clause.

Provided also that the inspector shall give the stop sale notice in writing to the person whose stocks have been detained and initiate appropriate action as per the provisions of this order within a period of twenty one days. If no action has been initiated by the inspector within the said period of twenty one days from the date of issue of the said notice, the notice of stop sale shall be deemed to have been revoked.

3. Where any fertiliser is seized by an inspector under this clause, he shall forthwith report the fact of such seizure to the collector whereupon the provisions of sections 6A, 6B, 6C, 6D and 6E of the Act, shall apply to the custody, disposal and confiscation of such fertilisers.

4. Every person, if so required by an inspector, shall be bound to afford all necessary facilities to him for the purpose of enabling him to exercise his powers under sub-clause (1).

VIII. ANALYSIS OF SAMPLES

29. Laboratory for analysis

1. A fertiliser samples, drawn by an inspector, shall be analyzed in accordance with the instructions contained in Schedule II in the - Central Fertiliser Quality Control and Training Institute, Faridabad or Regional Fertiliser Control Laboratories at Bombay, Madras or Kalyani (Kolkata) or in any other laboratory notified for this purpose by the State Government [with the prior approval of the Central Government.

   (1A) Biofertiliser samples, drawn by an inspector, shall be analyzed in accordance with the instructions contained in Schedule III in the – National Centres of Organic Farming, Ghaziabad or Regional Centres of Organic Farming at Bangalore, Bhubaneshwar, Hissar, Imphal, Jabalpur and Nagpur or in any other laboratory notified by the Central or State Government.

   (1B) Organic fertiliser samples, drawn by an inspector, shall be analyzed in accordance with the instructions contained in Schedule IV in the –National Centres of Organic Farming, Ghaziabad or Regional Centres of Organic Farming at Bangalore, Bhubaneshwar, Hissar, Imphal, Jabalpur and Nagpur or in any other laboratory notified by the Central or State Government.

2. Every laboratory referred to in sub-clause (1) shall, in order to ensure accurate analysis, of fertiliser samples, possess minimum equipment and other laboratory facilities, as may be specified from time to time by the Controller in this behalf

   29A. Qualifications for appointment of fertiliser analyst in the ferti1ser control laboratories

   No person shall be eligible for appointment as fertiliser analyst for analysis of fertiliser samples in the laboratories notified under clause 29 of the Order, unless he possesses the following qualifications, namely:-

   1. graduate in Agriculture or Science with chemistry as one of the subjects from a recognised university; and

   2.training In fertiliser quality control and analysis at Central Fertlllzer Quality Control and Training Institute, Faridabad. Provided that the fertiliser analysts appointed before the commencement of this Order, who do not possess the requisite training, shall undergo prescribed training, within a period of three years, in the Central Fertiliser Quality Control " and Training Institute, Faridabad from the date of commencement of this Order.

29B Laboratories for refree analysis

1.Every laboratory referred to in sub-clause (1) of clause 29 shall be designated as referee laboratory for the purpose of analysis of any sample of fertiliser :

Provided that no such laboratory which carried out the first analysis of the fertiliser sample shall be so designated in respect of that sample: Provided further that in respect of any sample the analysis of which has been challenged, may be sent for referee analysis to any one of the other laboratories except those which are located in the State or where the first analysis has been done.

Provided also that the Central Fertiliser Quality Control and Training Institute and Regional laboratories shall be considered as one group of laboratories and a sample first analysed by any one of them, shall not be sent for referee analysis to any other in that group, but only to any other laboratory notified by a State Government.

2. Not with standing anything contained in this Order, the Appellate Authority as specified under paragraph (b) of sub-clause (1) or paragraph (b) of sub-clause(2) of clause 32, in case of sample analyzed by the State Government laboratory, or the Controller, in case of samples analyzed by Central Fertiliser Quality Control and Training Institute, Faridabad or its Regional Fertiliser Control Laboratories, as the case may be, shall decide and send, one of the two remaining samples, for reference analysis as provided under sub-clause (1).

3.The Appelate authority as specified in sub clause 32 A or the Controller as the case may be shall on receipt of an appeal under sub clause 2 decide and send the third sample for analysis to any of the National Test house Laboratories at Chennai, Kolkata, Mumbai, Ghaziabad or Jaipur.

29 C.Laboratories for referee Analysis of Biofertiliser

1. National Centre of Organic Farming, Ghaziabad or Regional centre of Organic Farming at Bangalore, Bhubaneshwar, Hissar (shifted to Panchkula), Imphal, Jabalpur and Nagpur and every laboratory referred to in clause 29(1A) shall be designated as referee laboratory for the purpose of analysis of any sample of Biofertiliser
   Provided that no such laboratory which carried out the first analysis of fertilizer sample shall be so designated in respect of that sample.

   Provided further that in respect of any sample the analysis of which has been challenged may be sent for referee analysis to any one of the other laboratories except those which are located in the state or where the first analysis has been done,

   Provided that National Centre of Organic Farming, Ghaziabad and Regional Centre of Organic Farming at Bangalore, Bhubaneshwar, Hissar (shifted to Panchkula), Imphal, Jabalpur and Nagpur shall be considered as one group of laboratories and a sample first analysed by any one of them, shall not be sent for referee analysis to any other in that group, but only to any other laboratory notified by a State Government or Central Government.

2. Notwithstanding anything contained in this order, the Appellate Authority as specified in sub-clause 32A in case of sample collected by the state Government laboratory, or the Controller, in case of sample collected by National Centre of Organic Farming, Ghaziabad or Regional Centre of Organic Farming at Bangalore, Bhubaneshwar, Hissar (shifted to Panchkula), Imphal, Jabalpur and Nagpur, as the case may be, shall decide and send, one of the two remaining samples, for reference analysis as provided under sub-clause (1).

29D. Laboratories for referee analysis of Organic fertilizer

1. National Centre of Organic Farming, Ghaziabad or Regional Centre of Organic Farming at Bangalore, Bhubaneshwar, Hissar (shifted to Panchkula), Imphal, Jabalpur and Nagpur and every laboratory referred to in clause 29 (1A) shall be designated as referee laboratory for the purpose of analysis of any sample of Organic fertilizer, provided that no such laboratory which carried out the first analysis of fertilizer sample shall be so designated in respect of that sample.

   Provided further that in respect of any sample the analysis of which has been challenged, may be sent for referee analysis to any one of the other laboratories except those which are located in the state or where the first analysis has been done.

   Provided that National Centre of Organic Farming, Ghaziabad and Regional Centre of Organic Farming at Bangalore, Bhubaneshwar, Hissar (shifted to Panchkula), Imphal, Jabalpur and Nagpur shall be considered as one group of laboratories and a sample first analysed by any one of them, shall not be sent for referee analysis to any other in that group, but only to any other laboratory notified by a State Government or Central Government.

2. Notwithstanding anything contained in this order, the Appellate Authority as specified in sub-clause 1 of clause 32A in case of sample collected by the state Government laboratory, or the Controller, in case of sample collected by National Centre of Organic Farming, Ghaziabad or Regional Centre of Organic Farming at Bangalore, Bhubaneshwar, Hissar (shifted to Panchkula), Imphal, Jabalpur and Nagpur, as the case may be, shall decide and send, one of the two remaining samples, for reference analysis as provided under sub-clause (1).

30. Time limit for analysis, and communication of result 2

1. Where sample of a fertlliser has been drawn, the same shall be dispatched alongwith a memorandum in Form K and in case of Organic fertilizers and Biofertilisers in Form KI to the laboratory for analysis within a period of seven days from the date of Its drawal.

2. The laboratory shall analyse the sample and forward the analysis report in Form L and in case of Organic fertilizer and Biofertiliser in Form LI within [30 days] from the date of receipt of the sample in the laboratory to the authority specified in the said memorandum.

3. The authority to whom the analysis report is sent under sub-clause (2) shall communicate the result of the analysis to the dealer/manufacturer/Importer/pool handling agency from whom the sample was drawn within [15 days] from the date of receipt of the analysis report of the laboratory.

IX. MISCELLANEOUS

31 Suspension, Cancellation Or Debarment

1. A Notified Authority, registering authority, or as the case may be, the controller may, after giving the authorized dealer or the holder of certificate of registration or certificate of manufacture or any other certificate granted under this Order, an opportunity of being heard, suspend such authorization letter or certificate or debar the dealer from carrying on the business of fertilizer on one or more of the following grounds, namely:-

a. that the authorization letter or certificate of registration or certificate of manufacture, as the case may be, has been obtained by wilful suppression of material facts or by misrepresentation of relevant particulars:

b. that any of the provisions of this Order or any terms and condition of the Memorandum of Intimation or certificate of registration or the certificate of manufacture, as the case may be, has been contravened or not fulfilled:

Provided that while debarring from carrying on the business of fertiliser or canceling the certificate, the dealer or the certificate holder thereof may be allowed for a period of thirty days to dispose of the balance stock of fertilizers, if any, held by him:

Provided further that the stock of fertilizer lying with the dealer after the expiry of the said period of thirty days shall be confiscated.

2. Where the contravention alleged to have been committed by a person is such as would, on being proved, justify his debarment from carrying on the business of selling of fertilizer or, cancellation of authorization letter or certificate of registration or certificate of manufacture or any other certificate granted under this Order to such person the Notified Authority or registering authority or, as the case may be, the controller may, without any notice, suspend such certificate, authorization letter, as an interim measure:

Provided that the registering authority, Notified Authority or, as the case may be, the controller shall immediately furnish to the affected person details and the nature of contravention alleged to have been committed by such person and, after giving him an opportunity of being heard, pass final orders either revoking the order of suspension or debarment within fifteen days from the date of issue of the order of suspension:

Provided further that where no final order is passed within the period as specified above, the order of interim suspension shall be deemed to have been revoked without prejudice, however, to any further action which the registering authority, Notified

Authority or, as the case may be, the controller may take against the affected person under sub-clause (1).

3. Wherever an authorization letter or certificate is suspended, cancelled or the person is debarred from carrying on the business of fertiliser, the Notified Authority, registering authority, or as the case may be, the Controller shall record a brief statement of the reasons for such suspension or, as the case may be, cancellation or debarment and furnish a copy thereof to the person whose certificate or authorization letter has been suspended or cancelled or business has been debarred.

4. Wherever the person alleged to have committed the contravention is an industrial dealer, the Notified Authority may take action against the holder of such certificate of registration under sub-clause (1) and sub-clause (2):

Provided that where such certificate is suspended or cancelled, the Notified Authority shall, within a period of fifteen days from the date of issue of such order of suspension or cancellation, furnish to the controller also, besides sending the same to the person whose certificate has been suspended or cancelled, a detailed report about the nature of contravention committed and a brief statement of the reasons for such suspension or, as the case may be, cancellation:

Provided further that the controller, shall, in case of the order for suspension passed by the Notified Authority, on receipt of the detailed report and after giving the person an opportunity of being heard, pass final order either revoking the order of suspension or canceling the certificate of registration, within fifteen days from the date of receipt of the detailed report from the Notified Authority, failing which the order of interim suspension passed by the Notified Authority shall be deemed to have been revoked, without prejudice however, to further action which the controller may take against the holder of certificate under sub-clause (1):

Provided also that the order of cancellation passed by the Notified Authority shall remain effective as if it had been passed by the controller till such time the Controller, on receipt of the detailed report from the Notified Authority, and if deemed necessary, after giving the person a fresh opportunity of being heard, pass the final order either revoking or confirming the order of cancellation.

32. Appeals at Central Government level

1. In any State, where the fertiliser allocation is made by the Central Government under this Order and if the suspension or cancellation of authorization letter of the manufacturer and or pool handling agency or debarment of business, in any way, has an effect of dislocating the said allocation and if the Central Government is of the opinion that it is necessary or expedient so to do for maintaining the supplies, may direct the concerned State Government to furnish detailed report about the nature of contravention and a brief statement of the reasons for such suspension or cancellation and pass such order as it may think fit, confirming, modifying or annulling the order of State Government

   Provided that if the report called by the Central Government is not received from the State Government within a period of fifteen days from the date of issue of the

communication, the Central Government may decide the case without the report, on merit.

2. Any person aggrieved by the analysis report of Central Fertiliser Quality Control and Training Institute or its regional laboratories may appeal to the Controller for referee analysis of such sample within a period of 30 days from the receipt of analysis report.

Provided that the Controller may entertain an appeal after the expiry of said period of 30 days if it is satisfied that there was sufficient cause for not filing it within that period.

3.The referee analysis report received from the laboratory referred to in sub-clause (2) shall supersede the analysis report submitted by first laboratory and shall be treated as final;.

Provided that in case where the sample is declared as non-standard both in the first analysis report and referee analysis report but in different parameters or there is wide variation in the analysis report of first analysis and referee analysis, as the case may be, the aggrieved person may appeal to the controller for third analysis within thirty days from the date of receipt of the report of referee analysis on payment of such charges as may be required for such analysis.

4. The Controller after providing an opportunity to the aggrieved party of being heard may send the third sample for analysis to the Laboratory and specified under sub-clause (3) of clause 29 B.

5. The result of the third analysis referred to in sub-clause (4) shall supersede the first analysis and referee analysis report and shall be treated as final.".

32A. Appeal at the State Government level

1. The State Government shall, by notification in the Official Gazette, specify such authority as the Appellate authority before whom the appeals may be filed within 30 days from the date of the order appealed against by any person, except by an industrial dealer, aggrieved by any of the following Orders or action of registering authority or a Notified Authority, namely:-

i. Refusing to grant a certificate of manufacture for preparation of mixture of fertilisers or special mixture of fertilizers; or
ii. Suspending or canceling a certificate of manufacture; or
iii. Suspending or canceling authorization letter or debarring from carrying on the business of selling of fertilizer, or
iv. non-issuance of certificate of manufacture within the stipulated period;
v. non-issuance of amendment in authorization letter within the stipulated period.

2.Any person aggrieved by analysis report of fertilizer Testing laboratories notified by the State Government may appeal to the appellate authority appointed under sub-clause (1) for reference analysis of such sample within thirty days from the date of receipt of analysis report.

"Provided that the Appellate authority may entertain an appeal after expiry of said period of thirty days if it is satisfied that there was sufficient cause for not filing it within that period."

(3) The report of reference analysis received from referee laboratory shall supersede the analysis report submitted first laboratory and shall be treated as final:

Provided that in case where the sample is declared as non-standard both in the first analysis report and referee analysis report but in different parameters or there is wide variation in the analysis report of first analysis and referee analysis , as the case may be, the aggrieved person may appeal to the appellate authority for third analysis within thirty days from the date of receipt of the report of referee analysis on payment of such charges as may be required for such analysis.

(4) The appellate authority after providing an opportunity to the aggrieved party of being heard may send the third sample for analysis to the laboratory specified under sub-clause (3) of clause 29 B.

5. The result of the third analysis referred to in sub-clause (4) shall supersede the report of first analysis and referee analysis and shall be treated as final.

33. Grant of duplicate copies of [authorization letter or Certificate of manufacture] certificate of registrations, etc.

Where [authorization letter or] a certificate of registration or a certificate of manufacture or any other certificate granted or, as the case may be, renewed under this Order is lost or [defaced, the notified authority]registering authority or, as the case may be, the Controller may, on an_application made in this behalf, together with the fee prescribed for this purpose under clause 36, grant a duplicate copy of such certificate.

34. Amendment of certificate of registration

The Notified Authority, registering or controller, as the case may be, may, on application being made by the holder of an authorization letter, a certificate of registration or certificate of manufacture, together with the fee prescribed for the purpose under clause 36, amend an entry in such authorization letter, certificate of registration or certificate of manufacture as the case may be.

35. Maintenance of records and submission of returns, etc.

1. The controller may by an order made in writing direct the dealers. manufacturers/ importers, and pool handling agencies:-

a. to maintain such books of accounts, records, etc. relating to their business in Form 'N'. and
b. to submit to such authority, returns and statements in such form and containing such information relating to their business and within such time as may be specified in that order.

2. Where a person holds certificates of registration for retail sale and wholesale sale of fertilisers, he shall maintain separate books of accounts for these two types of sales made by him.

3. Where a State Government, a manufacturer, +an importer and a pool handling agency holds valid certificates of registration for sale of fertilisers in, wholesale or retail or both and also for sale for industrial use, he shall maintain separate books of accounts for these two or three types of sales made by him.

4. Every importer shall inform the Director of Agriculture of the State in which he intends to discharge the imported fertilizer, under intimation to the Central Government, before the import is made orwithin a period of fifteen days after an indent for import is placed, the following details, namely ;-

i. name of fertiliser
ii. name of country of import.
iii. name of manufacturer.
iv. quantity to be imported
v. date of arrival of the consignment.
vi. name of the discharge port.
vii. Name, designation of authorized or responsible person along with mobile number
viii.other information

36. Fees

1. The fees payable for grant, amendment or renewal of a[n authorization letter] or certificate of registration or certificate of manufacture a duplicate of such certificates or, renewal thereof under this Order shall be such as the State Government may, from time to time fix, subject to the maximum fees fixed for different purposes by the Central Government and different fees may be fixed for different purposes or for different classes of dealers or for different types of mixtures of fertiliser or special mixture.

2. The authority to whom and the manner in which the fee fixed under subclause (1) shall be paid, shall be such as may be specified by the State 26 Government by notification in the Official Gazette.

3. Any fee paid under sub-clause (1) shall not be refundable unless the grant or renewal of any certificate of registration or certificate of manufacture or duplicate copy of such certificate or renewal under this Order has been refused.

3. The fees payable for grant, amendment, renewal or duplicate copy of certificate of registration for industrial dealer and the authority to whom and the manner in which such fee shall be paid, shall be such as may be specified by the Controller from time to time by notification in the Official Gazette.

37. Service of orders and directions

Any order or direction made or issued by the controller or by any other authority under this order shall be served in the same manner as provided in sub-section (5) of section 3 of the Act.

38. Advisory Committee

1. The Central Government may by notification in the Official Gazette and on such terms and conditions as may be specified in such notification, constitute a Committee called the Central Fertiliser Committee consisting of a Chairman and not more than ten other persons having experience or knowledge in the field, who shall be members of the Committee, to advise the Central Government regarding:-
i. inclusion of a new fertiliser, under this Order;
ii. specifications of various fertilisers;
iii. grades/formulations of physical/granulated mixtures of fertilisers that can be allowed to be prepared in a State;
iv. requirements of laboratory facilities in a manufacturing unit, including a unit manufacturing physical/granulated mixtures of fertilisers;
v. methods of drawal and analysis of samples.
vi. any other matter referred by the Central Government to the Committee.

2. The Committee may, subject to the previous approval of the Central Government, make bye-laws fixing the quorum and regulating its own procedure and the conduct of all business to be transacted by it.

3. The Committee may co-opt such number of experts and for such purposes or periods as it may deem fit, but any expert so co-opted shall not have 27 the right to vote.

4. The Committee may appoint one or more sub-committees, consisting wholly of members of the Committee or or partly of the members of the Committee and partly of co-opted members as it thinks fit, for the purpose of discharging such of its functions as may be delegated to such subcommittee or sub-committees by the Central Fertiliser Committee.

5. The State Government may by notification in the Official Gazette and on such terms and conditions as may be specified in such notification, constitute a Committee called the State Fertiliser Committee consisting of a Chairman and not more than .4 other members, having experience or knowledge in the field, including a representative from State Agricultural University, the Fertiliser Industry and Indian Micro Fertilisers Manufacturers Association to advise the State Government regarding the grades/formulations of mixture or of fertilisers.

39. Repeal and saving

1. The Fertiliser Control) Order, 1957 is hereby repealed except as respects things done or omitted to be done under the said Order before the commencement of this Order.

2. Notwithstanding such repeal, an order made by any authority, which is in force immediately before the commencement of this Order and which is consistent with this Order, shall continue in force and all appointments made, prices fixed, certificates granted and directions issued under repealed Order and in force immediately before such commencement shall likewise continue in force and be deemed to be made, fixed, granted or issued in pursuance of this Order till revoked.

"Schedule III
[See clause 2(h) and (q)]
PART – A
Specification of Biofertilizers

1. Rhizobium

(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cell/g of powder, granules or carrier material or $1x10^8$ cell/ml of liquid.
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5
(v)	Particles size in case of carrier based material.	All material shall pass through 0.15-0.212mm IS sieve
(vi)	Moisture percent by weight, maximum in case of carrier based.	30-40%
(vii)	Efficiency character	Should show effective nodulation on all the species listed on the packet.

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

2. Azotobacter

(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cell/g of powder, granules or carrier material or $1x10^8$ cell/ml of liquid.
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5
(v)	Particles size in case of carrier based material.	All material shall pass through 0.15-0.212mm IS sieve
(vi)	Moisture percent by weight, maximum in case of carrier based.	30-40%
(vii)	Efficiency character	The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed.

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

3. Azospirillum

(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cell/g of powder, granules or carrier material or $1x10^8$ cell/ml of liquid.
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5
(v)	Particles size in case of carrier based material.	All material shall pass through 0.15-0.212mm IS sieve
(vi)	Moisture percent by weight, maximum in case of carrier based.	30-40%
(vii)	Efficiency character	Formation of white pellicle in semisolid N-free bromothymol blue media.

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

4. Phosphate solubilising Bacteria

(i)	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
(ii)	Viable cell count	CFU minimum $5x10^7$ cell/g of powder, granules or carrier material or $1x10^8$ cell/ml of liquid.
(iii)	Contamination level	No contamination at 10^5 dilution
(iv)	pH	6.5-7.5 for moist/dry powder, granulated carrier based and 5.0 – 7.5 for liquid based
(v)	Particles size in case of carrier based material.	All material shall pass through 0.15-0.212mm IS sieve

(vi)	Moisture percent by weight, maximum in case of carrier based.	30-40%
(vii)	Efficiency character	The strain should have phosphate solubilizing capacity in the range of minimum 30%, when tested spectrophotometrically. In terms of zone formation, minimum 5mm solubilization zone in prescribed media having at least 3mm thickness.

***Types of Carrier:-** The carrier material such as peat, lignite, peat soil, humus, wood Charcoal or **similar** material favouring growth of the organism.

5. Mycorrhizal Biofertilizers

i.	Form/base	Fine Powder/ tablets/ granules/ root biomass mixed with growing substrate
ii.	Particle size for carrier based powder formulations	90% should pass through 250 micron IS sieve (60 BSS)
iii.	Moisture content percent maximum	8 -12
iv.	pH	6.0 to 7.5
v.	Total viable propagules/ gram of product	100 gm of finished product with minimum 60 spres per gram
vi.	Infectivity potential	Inoculum potential : 1200 IP/g {determined by MPN method with 10 fold dilution}

6. Potassium Mobilizing Biofertilizers (KMB)

1.	Base	Carrier based* in form of moist/dry powder or granules, or liquid based
2.	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules, or carrier material on dry weight basis or $1x10^8$ cell/ml of liquid
3.	Contamination	No contamination at 10^5 dilution
4.	pH	6.5-7.5 for carrier based in form of powder or granules and 5.0-7.5 for liquid based
5.	Particle size in case of carrier based moist powder	Powder material shall pass through 0.15 to 0.212 mm IS sieve
6.	Moisture per cent, by weight, maximum in case of powder based	30-40
7.	Efficiency character	Maximum 10 mm solubilization zone in prescribed media having at least 3mm thickness.

*Type of carrier – The carrier material such as peat, lignite, peat soil, humus, talc or similar material favouring growth of microorganisms.

7. Zinc Solubilizing Biofertilizers (ZSB)

1.	Base	Carrier based in form of moist/dry powder or granules, or liquid based
2.	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules, or carrier material on dry weight basis or $1x10^8$ cell/ml of liquid
3.	Contamination	No contamination at 10^5 dilution
4.	pH	6.5-7.5 for carrier based in form of powder or granules and 5.0-7.5 for liquid based
5.	Particle size in case of carrier based moist powder	Powder material shall pass through 0.15 to 0.212 mm IS sieve
6.	Moisture per cent, by weight, maximum in case of powder based	30-40
7.	Efficiency character	Maximum 10 mm solubilization zone in prescribed media having at least 3mm thickness.

8. Acetobacter

1.	Base	Carrier based in form of moist/dry powder or granules, or liquid based
2.	Viable cell count	CFU minimum $5x10^7$ cells/g of powder, granules, or carrier material or $1x10^8$ cells/ml of liquid
3.	Contamination level	No contamination at 10^5 dilution
4.	pH	5.5-6.0moist/dry powder, granulated or carrier based and 3.5-6.0 for liquid

5.	Particle size in case of carrier based material	Al material shall pass through 0.15 to 0.212 mm IS sieve
6.	Moisture per cent, by weight, maximum in case of carrier based	30-40%
7.	Efficiency character	Formulation of yellowish pellicle in semisoilid medium N free medium

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

9. Carrier Based Consortia

1.	Base	Carrier based in form of moistpowder or granules
2.	Viable cell count	CFU minimum in a mixture of any 2 or maximum three of following microorganisms : CFU minimum Rhizobium or Azotobacter or Azospirillum $1x10^7$per g CFU minimum PSB $1x10^7$ per g CFU minimum KSB $1x10^7$per g
3.	Particle size in case of carrier based moist powder	Al material shall pass through 0.15 to 0.212 mm IS sieve
4.	Total viable count of all the biofertiliser organisms in the product	CFU minimum – $5x10^7$ cells per gm of carrier/ powder
5.	Moisture per cent, by weight, maximum in case of carrier based	30-40%
6.	Contamination	No contamination at 10^{-4} dilution for carrier based / granule based inoculants
7.	Efficiency character : Azotobacter Azospirillum PSB KMB Rhizobium	 The strain should be capable of fixing at least 10 mg of Nitrogen fixation/g of C-source The strain should be capable of fixing at least 10 mg of N-fixation/g of malate applied Minimum 5mm zone of solubilization zone on PSB media having at least 3mm thickness Minimum 5mm zone of solubilization on KSB media having at least 3mm thickness Nodulation test positive

10. Liquid Consortia

1.	Individual Viable count in Liquid based	CFU minimum in a mixture of any 2 or more of following microorganisms : CFU minimum Rhizobium or Azotobacter or Azospirillum $1x10^8$per ml CFU minimum PSB $1x10^8$ per ml CFU minimum KSB $1x10^8$per ml
2.	Total viable count of all the biofertiliser organisms in the product	CFU minimum – $5x10^8$ cells per ml of liquid based
3.	Contamination	No contamination at any dilution
4.	pH	5.0-7.0
5.	Efficiency character : Azotobacter Azospirillum PSB KMB Rhizobium	 The strain should be capable of fixing at least 10 mg N-fixation/g of C-source The strain should be capable of fixing at least 10 mg of N-fixation/g of malate applied Minimum 5mm zone of solubilization zone on PSB media having at least 3mm thickness Minimum 5mm zone of solubilization on KSB media having at least 3mm thickness Nodulation test positive

11. Phosphate Solubilizing Fungal Biofertilizer

Sl. no.	Components	Specifications
1.	Base	Carrier base in the form of moist/dry powder or granules or liquid based
2	Moisture percentage by weight maximum in case of carrier base	10
3	Spore count (per ml or gram)	Minimum $1x10^6$ spores/g
4.	contamination	Nil for liquid inoculums $1x10^3$ cells/gm for carrier base preparation
5	pH	Liquid: 3.5 to 5.5 Carrier :6.0 to 7.0
6.	Efficiency Character	The strain should have phosphate solubilization capacity in the range of 30%, when tested spectrometrically. In terms of zone Formation Minimum 10 mm Solubilization zone in prescribed media having least 3 mm thickness.

Part-B

Tolerance limit of Biofertilizers

1. In case of Rhizobium, Azotobacter, Azospirillum and Phosphate solubilizing bacteria, the total viable counts shall not be less than 1 x 10^7 CFU/gm of carrier material in the form of powder or granules or 5x 10^7 CFU/ml in case of liquid formulations during the entire period of shelf life.

2. In case of Mycorrhizal Biofertilizers, the viable propagules shall not be less than 80.

PART C

Procedure for drawl of samples of Biofertilizers

Procedure for sampling of biofertilizers

1. General Requirements of Sampling

1.0 In drawing, preparing and handling the samples, the following precautions and directions shall be observed.

1.1 Sampling shall be carried out by a trained and experienced person as it is essential that the sample should be representative of the lot to be examined.

1.2 Samples in their original unopened packets should be drawn and sent to the laboratory to prevent possible contamination of sample during handling and to help in revealing the true condition of the material.

1.3 Intact packets shall be drawn from a protected place not exposed to dampness, air, light, dust or soot."

2. Scale of Sampling

2.1 Lot

All units (containers in a single consignment of type of material belonging to the same batch of manufacture) shall constitute a lot. If a consignment consists of different batches of the manufacture the containers of the same batch shall be separated and shall constitute a separate lot.

2.2 Batch

All inoculant prepared from a batch fermentor or a group of flasks (containers) constitute a batch.

2.3 For ascertaining conformity of the material to the requirements of the specification, samples shall be tested from each lot separately.

2.4 The number of packets to be selected from a lot shall depend on the size of the lot and these packets shall be selected at random and in order to ensure the randomness of selection procedure given in IS 4905 may be followed."

3. Drawal of Samples

3.1 The Inspector shall take three packets as sample from the same batch. Each sample constitutes a test sample.

3.2 These samples should be sealed in cloth bags and be sealed with the Inspector's seal after putting inside Form P. Identifiable details such as sample number, code number or any other details which enable its identification shall be marked on the cloth bags.

3.3 Out of the three samples collected, one sample so sealed shall be sent to in-charge of the laboratory notified by the State Government under clause 29 or to National Centre for Organic Farming or to any of its Regional Centres. Another sample shall be given to the manufacturer or importer or dealer as the case may be. The third sample shall be sent by the inspector to his next higher authority for keeping in safe custody. Any of the latter two samples shall be sent for referee analysis under subclause (2) of clause 29B.

3.4 The number of samples to be drawn from the lot

Lot/Batch	**Number of Samples**
Upto 5,000 packets	03
5,001-10,000 packets	04
More than 10,000 packets	05

Schedule III
Part D
METHODS OF ANALYSIS OF BIOFERTILIZERS

1. A. METHODS OF ANALYSIS OF RHIZOBIUM BIOFERTILISERS

1. Apparatus

1.1 Pipettes Graduated 1 ml and 10 ml.

1.2 Dilution Bottles or flasks

1.3 Petri Dishes Clear, Uniform, flat-bottomed.

1.4 Hot Air Oven - Capable of giving uniform and adequate temperature, equipped with a thermometer calibrated to read up to 250^{o} C and with vents suitably located to assure prompt and uniform heating.

1.5 Autoclave

1.6 Incubator

1.7 Hand Tally or Mechanical counting Device

1.8 pH meter

2. Reagents

2.1 Congo Red-one percent aqueous solution

2.2 Medium

Use a plating medium of the following composition:

Agar	20 g
Yeast Extract	1 g
Mannitol	10 g
Potassium hydrogen phosphate (K_2HPO_4)	0.5g
Magnesium sulphate ($MgSO_4$ $7H_2O$)	0.2g
Sodium Chloride (NaCl)	0.1g
Congo red	2.5 ml
Distilled water	1000 ml
pH	7.0

2.3 Sterilizing and preparation procedure for plates:

2.3.1. Sterilize the sampling and plating equipment with dry heat in a hot air oven at not less than 160^{o} C for not less than 2 hours.

2.3.2 Sterilize the media by autoclaving at 120^{o} C for 20 min. To permit passage of steam into and from closed container when autoclaved, keep stoppers slightly loosened or plugged with cotton. Air from with in the chamber of the sterilizer should be ejected allowing steam pressure to rise.

Preparation of Plating Medium and Pouring

1.3.3 Prepare growth medium in accordance with the composition of the specific Biofertilizers.

1.3.4 Melt the required amount of medium in boiling water or by exposure to flowing steam in partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Melt enough medium which will be used with in 3h. Re-sterlization of the medium may cause partial precipitation of ingredients.

1.3.5 When holding time is less than 30 min promptly cool the molten medium to about 45ºC, and store until used, in a water bath or incubator at 43ºC to 45ºC. Introduce 12 to15 ml of liquefied medium or appropriate quantity depending on size of the Petri dish at 42 to 44º C into each plate. Gently lift the cover of the dish just enough to pour in the medium. Sterlize the lips of the medium container by exposure to flame.

a. Immediately before pouring.
b. Periodically during pouring, and
c. When pouring is completed for each batch of plates, if portion of molten medium remain in containers and are to be used without subsequent sterilization for pouring additional plates. As each plate is poured thoroughly mix the medium with test portions in the Petri dish.

2.3.6 By rotating and tilting the dish and without splashing the medium over edge, spread the medium evenly over the bottom of the plate. Provide conditions so that the medium solidifies with reasonable promptness (5-10min) before removing the plates from surface.

3. Preparation of serial dilution for plate count

3.1. Dispense 30 g of Inoculants to 270 ml of sterile distilled/ demineralized water and shake for 10 min on a reciprocal shaker or homogenizer. Make serial dilutions up to 10^{-9} by suspending 10 ml aliquot of previous dilution to 90ml of water. Take 0.1 ml or suitable alliquotes of 10^{-5} to 10^{-9} dilutions using sterile pipettes and deliver to Petri dishes containing set medium as given in 2.1 and spread it uniformly with a spreader. Invert the plates and promptly place them in the incubator.

4. Incubation of plates

4.1. Label the plates and incubate at 28±2º C for 3 to 5 days for fast growing Rhizobia and 5 to 10 days for slow- growing ones.

4.2. Colony counting aids

Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in Centimeter Square. Record the total number of colonies with the hand tally. Avoid mistaking particles of undissolved medium or precipitated matter, in plates for pinpoint colonies. To distinguish colonies from dirt, specks and other foreign matter, examine doubtful objects carefully.

4.3. Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies, which absorb Congo red and stand out as reddish colonies. Rhizobium stands out as white, translucent, glistening and elevated colonies. Count such colony numbers and calculate figures in terms of per gram, of carrier. Also check for freedom from contamination at 10^{-5} dilution.

5. Test for nodulation

5.1 Pot culture test

Plant nutrient solution

Sl. No	Composition	Conc.	g/l
a	Potassium chloride	0.001M	0.0745
b	Di Potassium hydrogen Phosphate (K_2HPO_4)	0.001M	0.175
c	Calcium sulphate ($CaSO_4$ $2H_2O$)	0.002M	0.344
d	Magnesium sulphate ($MgSO_4$ $7H_2O$)	0.001M	0.246
e	Trace elements solution:	-	0.5ml
	1. Copper sulphate ($CuSO_4$ $5H_2O$) 2. Zinc Sulphate ($ZnSO_4$ $7H_2O$) 3. Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$ $4H_2O$) 4. Magnesium sulphate ($MgSO_4$ $7H_2O$) 5. Boric acid (H_3BO_4) 6. Water	0.01mg/kg 0.25 mg/kg 0.0025 mg/kg 0.25 mg/kg 0.125 mg/kg	0.78 2.22 0.01 2.03 1.43 1 lit
	Prepare the solution no (e) consisting of trace elements in one liter of stock solution and add to final nutrient solution at the rate of 0.5 ml per liter.		
f	Iron solution:		0.5 ml
	Ferrous sulphate Citric acid Water		5 5 100 ml
	Prepare the solution no. (f) As 100 ml of stock solution and add final nutrient solution at the rate of 0.5 ml per liter.		

5.2 Preparation

Prepare the nutrient solution by weighing out substances (a), (b) and (d) and dissolving them in a liter of water. To this solution add 0.5 ml of trace elements solution and 0.5 ml of iron solution. Grind in a mortar 0.344 g of calcium sulphate (c) to a fine consistency and add to the final nutrient solution. Auto clave the nutrient solution thus prepared, at 120°C for 20 min.

Notes

1. The nutrient solution may be prepared in the tap water provided the water is soft.
2. The nutrient solution should be shaken well to disperse calcium sulphate before dispensing.
3. If the solution is made up with distilled water, the pH is about 7.2 before autoclaving and falls to 5.5 on autoclaving and rises slowly on standing to about 5.8. However, there is no need to adjust pH. For most tropical legumes; pH of about 6.0 is adequate.

5.3 Procedure

5.3.1 Immerse the seeds in 95 percent alcohol and follow by surface sterilization in freshly prepared chlorine water (for 15 to 20 min) or 0.1 percent mercuric chloride solution 3 min in a suitable container such as a screw – capped bottle or a test tube with a rubber hung. In case of seeds with tough seed coat, concentrated sulphuric acid may be used as a surface Sterilants for 20 to 30 min. It is recommended that the seeds should be placed overnight in a desiccators containing calcium chloride before surface sterilization with sulphuric acid. Pour out the Sterilants and wash the seeds in several changes of sterile water and wash the seeds in several changes of sterile water (at least ten times) to get rid of the Sterilants. Fill earthenware or glazed pot with soil (2 parts soil and 1 part

washed coarse sand) (pH 6 to 7) and autoclave for 2 h at 120° C. After two days incubation at room temperature, repeat autoclaving to ensure complete sterility of soil. Inoculate surface sterilized seeds with water slurry of the inoculant taken from a culture packet (15 to 100 g seeds per gram of inoculants depending on the size of the seed) and sow the seeds. Keep a set of pots with Uninoculated seeds as control and also a set of pots with ammonium nitrate at the rate of 100 kg N/ha as control aid incubate them in a pot-culture house during appropriate seasons for appropriate plants, taking care to separate the inoculated pots from the control pots. If growth rooms or cabinets having facilities to adjust temperature and light are available, the pots may be incubated in such controlled environmental conditions. Sterilize the nutrientsolution at 120° C for 20 min and irrigate each potonce to the moisture holding capacity of soil. Subsequently, water the seedlingperiodically with sterilized water preferably through a plastic tube, taking care to prevent splashing of water from inoculated pots to uninoculated ones. Maintain required number of replicated pots (4 to 6) for each botanical species for statistical analysis.

5.3.2 After two to three weeks of growth, thin down the number of plants in each pot to four uniform plants. At the end of 6 to 8 weeks, take one set of pots from both the control and inoculated series and, separate the plants carefully from the soil under slow running water. Obtain data on the number, colour (effective nodules are pink or red) and mass of nodules. At the end of 6 to 8 weeks, harvest the shoot system, dry at 60° C for 48 h and determine dry mass. For the above purpose, maintain adequate replications of pots (4 to 16).

5.3.3 Record the nodulation data regarding formation of pink colour of nodules as revealed visually when nodules are cut open by razor blade. After computing the data, based on the dry mass of plants and nodulation data decide the effectiveness of culture. If good effective pink nodulation is obtained in inoculated plants together with local absence or sometimes presence of stray nodules in controls and if there is a 50 percent increase in the dry mass of plants over the Uninoculated control without nitrate, it may be concluded that the culture is of the require quality.

1.B. METHOD OF ANALYSIS OF AZOTOBACTER BIO-FERTILISER

1. Apparatus - same as Rhizobium

2. Reagents:

2.1. Medium

Use a plating medium of the following composition

Agar	20g
Sucrose (C_{12} H $_{22}O_{11}$)	20.0 g
Ferric sulphate Fe_2 (SO_4) $_3$	0.1g
Dibasic potassium phosphate (K $_2HPO_4$)	1.0g
Magnesium sulphate ($MgSO_4$, 7H $_2$O)	0.5g
Sodium Chloride (NaCl)	0.5g
Calcium carbonate ($CaCO_3$)	2.0g
Sodium Molybdate (Na $_2$MoO4)	0.005gms
Distilled water	1000ml
pH	6.8 to 7.2

2.2. Sterilization and preparation procedure for plates:Same as Rhizobium

2. Preparation of plating medium and pouring

Preparation of serial dilution for plate counts

3.1. Dispense 30 g of Inoculants to 270 ml of sterile distilled/ demineralized water and shake for 10 min on a reciprocal shaker or homogenizer. Make serial dilutions up to 10^{-9} by suspending 10 ml aliquot of previous dilution to 90ml of water. Take 0.1 ml or suitable aliquotes of 10^{-5} to 10^{-9} dilutions using sterile pipettes and deliver to Petri dishes containing set medium as given in 2.1 and spread it uniformly with a spreader. Invert the plates and promptly place them in the incubator.

4. Incubation of plates: - Same as Rhizobium.

4.1. Label the plates and incubate at 28+/- 3^{o} C for 4 to 6 days.

4.2**.** Colony counting aids: Same as Rhizobium.

Azotobacter chroococcum colonies are gummy, raised with or without striations, viscous and often sticky. The pigmentation varies from very light brown to black. Count the colony number and observe the cyst formation as given below and calculate number per gram of the carrier material.

Grow the vegetative cells at 30^{o} C on Burks agar medium comprising sucrose 20 g, dipotassium hydrogen phosphate 0.64 g, dihydrogen potassium phosphate 0.20 g, sodium chloride 0.20 g, calcium sulphate 0.05 g, sodium molybdate0.001 g, ferric sulphate 0.003 g, agar 20 g and distilled water 1000 ml. Look for vegetative cells after 18 to 24 h either by simple staining method or through a phase contrast microscope.

Grow the cyst cells on Burks agar medium as given above with 0.3 percent n-butanol in place of the carbon source. Look for cyst formation after 4 to 5 days incubation.

5. Test for Nitrogen fixation in pure cultures

5.1. Pure culture medium

5.1.1. Prepare medium as given for Azotobacter (2.1 under 1B), excluding agar.

5.2. Procedure

Select from each Azotobacter colony, of the type that has been counted as Azotobacter chroococcum.Pick up one colony and plate on the medium given in. Use this pure culture for inoculating the broth for nitrogen fixation. For this purpose, take 50 ml aliquots of broth in 250 ml conical flasks for inoculation. After 12 days growth at 28^{o} C, test the contents of the flasks for purity by streaking on fresh medium and concentrating over water bath (50 to 60^{o} C) to dryness. Wash the dried culture and take it as a sample. The contents of the flasks in inoculated control series should be similar manner.

5.3. Determination by Kjeldahl Method

(i) Reagents

(ii) Sulphuric acid-93-98 percent, N-free

(iii) Digestion mixture- Mix copper sulphate and potassium sulphate in the ratio 1: 10 and grind them to a fine powder.

(iv) Sodium hydroxide pellets or solution, N-free- For solution, dissolve about 450 g of sodium hydroxide in water, cool, and dilute 1 liter (sp gr of the solution should be at least 1.36)

(v) Zinc granules-reagent grade.

(vi) Indicators: -

(a) Methyl red indicator - Dissolve 1g of methyl red in 200ml of Ethanol.

(b) Mixed indicator - Prepare mixed indicator by Dissolving 0.8 of methyl red and 0.2 g of methyl blue in 500 ml of ethanol.

(vi) Hydrochloric or sulphuric acid – Standard solution 0.5 or 0.1 N when amount of nitrogen is small.

(vii) Sodium hydroxide standard solution – 0.1 N (or other specified concentration)

Note : Ratio of salt to acid (m/v) should be about 1:1 at the end of the digestion for proper temperature control. Digestion may be incomplete at a lower ratio, and nitrogen may be lost at higher ratio. Each gram of fat consumes 10 ml of sulphuric acid and each gram of carbohydrate 4.0 ml of sulphuric acid during digestion.

5.4. Apparatus

(i) For digestion - Use kjeldahl's flasks of hard, moderately thick, well annealed glass with total capacity approximately 500 to 800 ml. Conduct digestion over heating device adjust to bring 250 ml of water at 25^{o} C to rolling boil in about 5 minutes. To test the heaters, preheat for 10 minutes in the case of gas burners and for 30 minutes in the case of electric heaters. Add 3 to 4 boiling chips to prevent superheating.

(ii) For distillation - Use 500 to 800ml kjeldahl's flask fitted with rubber stopper through which passes the lower end of an efficient scrubber bulb or trap to prevent mechanical carry-over of sodium hydroxide during distillation. Connect the upper end of the bulb tube to a condenser by rubber tubing. Trap the out let of the condenser in such a way as to ensure absorption of ammonia distilled over with the receiver.

1.5. Procedure: -

(a) Place 0.25 g of the sample in the digestion flask. Add 0.7 gm mercuric oxide, 15 gm potassium sulphate followed by 25 ml of sulphuric acid. Shake, let stand for about 30 minutes and heat carefully until frothing ceases. Boil briskly until the solution clears and continue boiling further for 90 minutes. Cool, add about 200 ml of water cool to room temperature and add a few zinc granules.

(b) Tilt the flask and carefully add 50 ml of sodium hydroxide solution without agitation. Immediately connect the flask to the distillation bulb on the condenser whose tip is immersed in 50 ml of standard 0.1 N acid in the receiving flasks.Rotate the digestion flask carefully to mix the content. Heat until 150 ml of the distillate collects and titrate excess acid with 0.1 N base using methyl-red or mixed indicator. Carry out blank determination on reagents.

Note: Check the ammonia recording periodically, using inorganic nitrogen control, for example, ammonium sulphate.

(c) Calculation: -

(i) Nitrogen content, percent by mass =

$$\frac{\text{(Milliliters of 0.1 N acid for sample – milliliters of 0.1 N acids for blank) X0.14}}{\text{Mass of sample taken}}$$

(ii) Total nitrogen in culture = Total dry mass of sample X percent nitrogen.

(d) Take a 1.0 g of accurately weighed sample each from the inoculated series and from the controls. Put them separately in 250 ml volumetric flask, add 150 ml water, mix the content and make up the volume to 250 ml water. Shake for 5 minutes and centrifuge for 15 minutes at 10000 rpm. Estimate glucose in the supernatant in triplicate. The difference between the two provides the data of actual amount of glucose consumed. Calculate the amount of nitrogen fixed per gram of sucrose consumed.

(i) **Determination of Glucose**: - From the supernatant, draw suitable aliquots and estimate reducing sugars (glucose) as follows:

Reagents.

(ii) Soxhelt modification of Fehling solution: - Prepare by mixing equal volumes of solution A and solution B immediately before using.

(iii) Copper sulphate solution (Solution A)- Dissolve 34.639 g of copper sulphate crystals (CuSO4 5H2O) in water, dilute to 500ml and filter through glass wool or filter paper.

Standardization of copper sulphate solution: - Using separate pipettes, pipette accurately 5 ml of solution A and 5 ml of solution B into a conical flask of 250 ml capacity. Heat this mixture to boiling on asbestos gauze and add standard invert sugar solution from a burette, about 1 ml less than the expected volume, which will reduce the Fehling solution completely (about 48 ml). Add 1 ml of methylene blue indicator while keeping the solution boiling. Complete the titration within 3 min, the end point being indicated by change of colour from blue to red. From the volume of invert sugar solution used, calculate the strength(s) of the copper sulphate solution by multiplying the titre value by 0.001 (mg/ml of the standard invert sugar solution). This would give the quantity of invert sugar required to reduce the copper in 5 ml of copper sulphate solution.

(iv) Potassium sodium tartrate (Rochelle salt) solution (solution B): - Dissolve 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in water, and dilute to 500 ml. Let the solution stand for a day, and filter.

(v) Hydrochloric acid – sp gr 1.18 at 20^{o} C (approximately 12 N)

(vi) Standard invert sugar solution –Weigh accurately 0.95 g of sucrose and dissolve it in 500 ml of water. Add 32 ml of concentrated hydrochloric acid, boil gently for 30 min and keep aside for 24 hours. Neutralize with sodium carbonate and make the final volume to 1000ml; 50 ml of this solution contains 0.05 g of invert sugar.

(vii) Methylene blue indicator- 0.2 percent in water.

(viii) Procedure: - Place about 1 g(M), accurately weighed, of the prepared sample of AI into a 250ml volumetric flask and dilute with about 150 ml of water. Mix thoroughly the contents of the flask and make the volume of 250 ml with water. Using separate pipettes, take accurately 5 ml each of solution A and solution B in a porcelain dish. Add about

12 ml of AI solution from a burette and heat to boiling over an asbestos gauze. Add 1 ml of methylene blue indicator and while keeping the solution boiling complete the titration within 3 minutes, the end point being indicated by change of colour from blue to red. Note the volume (H) in ml of AI solution required for the titration.

(ix) Calculation

Total reducing sugars, percent by mass = $\frac{250 \times 100 \times S}{H \times M}$

Where
S = strength of copper sulphate solution,
H = volume in ml of AI solution required for titration, and
M = mass in g of AI taken for the test.

5.6 Determination of sucrose

(i) Procedure: - To 100 ml of the stock AI solution, add 1 ml of concentrated hydrochloric acid and heat the solution to near boiling. Keep aside overnight. Neutralize this solution with sodium carbonate and determine the total reducing sugars as described in.

(ii) Calculation

(a) Sucrose, percent by mass = (reducing sugars after inversion, percent by mass) –(reducing sugars before inversion, percent by mass) x 0.95

(b) Nitrogen, mg per gram of sucrose consumed = 2(a-b)-C
Where
a= initial quantity of sucrose taken for the test
b=mass of sucrose as calculated in (a),and
c= amount of nitrogen fixed per gram of glucose.

1.C. Method of Analysis of Azospirillum Biofertilisers

1.**Apparatus*:*** same as Rhizobium

2.**Reagents**

2.1 **Medium**

Use N-free semisolid medium (Nfb) of the following composition for preparation of MPN tubes

DL-Malic acid	5.0
K_2HPO_4	0.5
$MgSO_4\ 7H_2O$	0.2
NaCl	0.1
$CaCl_2$	0.02
Trace element Soln.	2.0 ml
Fe EDTA (1.64% Soln.)	4.0 ml
Vitamin soln.	1.0 ml
KOH	4.0 ml
Bromothymol blue (0.5% aq.)	2.0 ml
Adjust pH to 6.8-7.0 with KOH	
For semi solid add agar	1.75 g
For solid medium add agar	15.0 g

2.1.1**Trace element solution (g/litre)**

$Na_2MoO_4\ 2H_2O$	0.2
$MnSO_4\ H_2O$	0.235
H_3BO_3	0.28

$CuSO_4 5H_2O$	0.008
$ZnSO_4\ 7H_2O$	0.024
Distilled water	1000 ml

Use 2 ml of this solution in one litre of Nfb media

Vitamin solution (g/litre)

Biotin	0.01
Pyridoxin	0.02
Distilled water	1000 ml

Use one ml of this sol. in one litre of Nfb media

2.2 **Sterilization and preparation of MPN tubes**

2.2.1 Prepare Nitrogen free Bromothymol Blue malate medium as mentioned at paragraph 2.1. Boil to dissolve agar. Quickly dispense 10 ml molten media in 15 x 150 ml test tubes or screw capped culture tubes and close either with cotton plugs or screw caps. Minimum of 25 such tubes shall be needed for each sample.

2.2.2 Sterilize the tubes by autoclaving at 121°C for 20 minutes, as in Rhizobium at paragraph 2.3.2.

3.**Preparation of serial dilution for MPN count**

Dispense 30 g of Azospirillum biofertilizers in 270 ml of sterile water and shake for 10 minutes on a reciprocal shaker. Make serial dilutions up to 10^{-8} dilution. Pipette out 1 ml aliquots of 10^{-4} to 10^{-8} dilution and deliver it to screw cap tubes or test tubes containing N-free semi solid Nfb media.

4.**Incubation of tubes**

Label the tubes and incubate at 36 $\pm 1^0$C for 3-4 days in vertical position in a test tubes stand. Do not disturb the medium during the entire period of incubation.

Table 1

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	--

*Most Probable Numbers for use with 10 fold dilution and 5 tubes per dilution (Cochran, 1950)

5.**Counting**

5.1 Count the tubes which have turned blue and have developed typical white sub-surface pellicle.

5.2 Count the tubes as +ve or –ve for the presence of sub-surface pellicle and consider for the purpose of calculation.

5.3**Method for Estimating MPN Count**

5.3.1 To calculate the most probable number of organisms in the original sample, select as P_1 the number of positive tubes in the least concentrated dilution in which all tubes are positive or in which the greatest number of tubes is +ve, and let P_2 and P_3 represent the numbers of positive tubes in the next two higher dilutions.

5.3.2 Then find the row of numbers in Table 1 in which P_1 and P_2 correspond to the values observed experimentally. Follow that row of numbers across the table to the column headed by the observed value of P.

5.3.3 The figure at the point of intersection is the most probable number of organisms in the quantity of original sample represented in the inoculum added in the second dilution. Multiply this figure by the appropriate dilution factor to obtain the MPN value.

5.3.4 Azospirillum count/g of carrier = $\frac{\text{Value from MPN table* x Dilution level}}{\text{Dry mass of product}}$

1.D. METHOD OF ANALYSIS OF PHOSPHATE SOLUBULISING BACTERIAL BIOFERTILIZER

1. **Apparatus** - Same as Rhizobium
2. **Reagents**

2.1 Medium

Use a plating medium of the following composition:

Glucose	10.0g
Tri-calcium phosphate	5.0g
Ammonium sulphate	0.5 g
Magnesium sulphate	0.1 g
Sodium Chloride	0.2 g
Yeast extracts	0.5 g
Manganese sulphate	Trace
Ferrous sulphate	Trace

Distilled water	1000ml
Agar	15.0 g
pH adjusted to	7 ± 0.2

2.2 Sterilizing & preparation procedure for plates:
Same as Rhizobium

2.3 Preparation of Plating Medium and Pouring
Same as Rhizobium

3. **Preparation of Serial Dilution for Plate Counts**:
Same as Rhizobium.

4. **Incubation of Plates**:
4.1. Label the plates and incubate at 28± 1° C for 4 to 6 days.
4.2 Colony counting aids: Same as Rhizobium

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

Methods of counting solubilisation zones
a. Take 10 g of PSBI (BF) in 90 ml in water
b. Make a ten fold dilution series up to 10^7.
c. Take 0.2 ml aliquote of 10^5 to 10^7 dilutions using sterile pipettes and delivered to petri dishes containing Pikowskya media.
d. Spread it uniformly, Invert the plates and incubate them up to 2 weeks at 28 ±2°C.
e. Count the colonies showing hallow cones and measure their diameter.

5. Determination of soluble phosphorus using Ascorbic acid method:

5.1 Apparatus
Spectrometer capable of transmission measurement at 840 to 880 mm.
Extractant: It is Olsen extract.

5.2 Reagents
Ammonium Molybdate [$(NH_4)_6\ Mo_7O_{24}.4H_2O$]
L- Ascorbic Acid
p-Nitro phenol
$4NH_2SO_4$

5.3 Preparation of reagents

5.3.1 Sulphomolybdic Acid:-
- Take 20 g of ammonium molybdate and dissolve in 300 ml of distilled water.
- Add slowly 450 ml of 10N - H_2SO_4.
- Cool the above mixture and add 100 ml of 0.5 percent solution of antimony Potassium tartrate.
- Cool and make the volume to one liter. Store in glass bottle away from direct sunlight.

5.3.2 Preparation of Mixed Reagent
Add 1.5 g of L-ascorbic acid in 100ml of the above stock solution and mix. Add 5ml of this solution to develop colour. Mixed reagent is to be prepared fresh as it does not keep for more than 24 h.

5.3.3 Procedure

"(i)Preparation of Sample

Pure culture medium same as at 2.1 above excluding agar.

Prepare broth medium in 100 ml aliquots in 6 no., 250 ml conical flasks and sterilize in autoclave at 121^0C for 20 min.

(ii) Inoculation of Medium

Select one PSB colony of the type that has been counted as PSB (showing sufficient zone of solubilization) and streak on set medium as described at 2.1 in a Petri dish. Use this pure culture for inoculating the broth. Inoculate 3 flasks and keep 3 flasks as uninoculated control. Incubate the flasks over rotary shaker for 12 days at $28+1^0$C.

After 12 days, filter the contents of each flask separately through Whatman No. 42 filter paper or centrifuge at 10,000 rpm for 15 min.

(iii) Add 10 ml of filtrate/ centrifugate to 50 ml of olsen extractant and shake for 30 min over rotary shaker.

(iv) Filter the suspension through Whatman filter paper No. 40. If the filtrate is coloured then add a tea spoon of Dacro-60 (activated phosphorous free carbon), reshake and filter.

(v) Take a known aliquot (5 to 25 ml) of the extract in a 50 ml volumetric flask.

(vi) Add 5 drops of p-nitrophenol indicator (1.5 per cent solution in water) and adjust the pH of the extract between 2 and 3 with the help of $4NH_2SO_4$. The yellow colour will disappear when the pH of the solution becomes 3. Swirl gently to avoid loss of the solution along with the evolution of CO_2.

(vii) When the CO_2 evolution has subsided, wash down the neck of the flask and dilute the solution to about 40 ml.

(viii) Add 5 ml of the sulphomolybdic acid mixed reagent containing ascorbic acid, swirl the content and make up the volume.

(ix) Measure the transmission after 30 min at 880 nm using red filter. The blue colour developed remains stable upto 60 minutes.

(x) Record the concentration of phosphorous (P) in the extract form the standard curve and calculate the concentration of soluble phosphorous as follows:"

5.3.4. Calculations

(a) Weight of the substance taken = x g
(b) Volume of the extract added = 50 ml
(c) Volume of the extract taken for P determination = y ml
(d) Volume made after colour developed = 50 ml
(e) Reading from the standard curve against percent transmission recorded = z ppm
(f) Soluble Phosphorous percent p = $\frac{Z \times 50 \times 10^{-6} \times 50 \times 100}{Y. X}$

5.3.4 Preparation of standard Curve

Prepare standard curve using 0.1 to 0.6 ppm P in 50 ml volumetric flask. Plot the standard curve by taking concentration of soluble P on x- axis and

percent T on y- axis using a semi log graph paper. It is a straight line relationship between the soluble P and percent T when plotted on a semi-log graph paper.

1. E. METHODS OF ANALYSIS FOR MYCORRHIZAL BIOFERTILIZERS

1. **Estimation of pH**
   As per methodology specified in Schedule IV Part D at Serial Number 1

2. **Estimation of moisture contents**
   As per methodology specified in schedule IV Part D at serial number of FCO 1985

3. **Estimation of total viable propagules**
   3.1. Harvesting of spores from finished product

   (a) **By sieving**

   Equipment and Reagent
   Stalking sieves with nylon or stainless steel mesh and a large range of pore sizes for isolating spores from the carrier or soil sample

   - 40-50 micron (0.04 mm) seive for small sized spores
   - 100 micron (0.10 mm) sieve for medium sized spores
   - 250 micron (0.25 mm) sieve for very large spores and sporocarps
   - Wash bottles containing water
   - Jars for collecting the sieving
   - Stereo zoom (stereomicroscope)
   - Petri dishes (11 cm) for observing the sieving under stereomicroscope
   - Micropipettes for spore picking
   - Centrifuge

   b. **Procedure** - Mix the Soil in a substantial volume of water and decant through a series of sieves arranged in descending order of mesh size. Roots and coarse debris are collected on a coarse (60-ISS) sieve, while spores are captured on one or more finer sieves. Vigorous washing with water is necessary to free spores from aggregates of clay or organic materials. Collect the sieving in jars. Transfer the sieving onto the grided petri dishes/plate and observe under stereomicroscope. Count the number of spores in plate/dish and express it as spores/g of the sample.

   c. By sucrose gradient

   i. Collect the sieving by the method described above. Transfer the sieving into centrifuge tubes and centrifuge for 5 minutes at 1750 rpm in a horizontal rotor.

   ii. Decant the supernatant liquid carefully and resuspend pellet in 60% sucrose solution. Again centrifuge for 2 –5 minutes

   iii. Pour the supernatant (with spores) onto a 300BSS sieve size and rinse with water to remove the sugar. Transfer the sieving onto the grided petri dishes/plate and observe under stereomicroscope. Count the number of spores in plate/dish and express it as spores/g of the sample.

3.2. Spore staining

a. Equipment and Reagent

1. Equipments and reagents for spore extraction as described previously.
2. 2,5-diphenyl-2N-tetrazolium bromide (MTT).
3. Distilled water
4. Eppendorf
5. Stereomicroscope
6. Petri-dishes

b. Procedure

- Prepare 0.25% solution of MTT (2,5-diphenyl-2N-tetrazolium bromide.
- Avoid exposure of MTT solution to light, as the stain is light sensitive.
- Add freshly collected AMF spores (approximately 100 in number) collected by any of the two methods described above, to the staining solution and incubate at 27^0C in sterile eppendorf in dark.
- Observe the spores for different colour reactions using stereomicroscope under dark field after 24 hours, 48 hours and 72 hours of incubation.
- Spores, which stained red or pink, are treated as viable.

$$\%\text{Spore viability} = \frac{\text{No. of spores which stained red or pink} \times 100}{\text{Total number of spores}}$$

4. Assessment of Infectivity Potential

The bioassay is used to determine the number of infective propagules present in the product. Once the infective propagules (spores, mycelia and vesicles in the root fragments) come in contact with the host roots they give out a turgid mycelial structure - the appressoria, which is the initial step in the penetration event. This appressoria enters the root through an 'entry point'. This entry point can be visualized by staining and enumerated as a measure of the infectivity of the inoculum. Host plants are grown from pre germinated seeds and a known weight of the inoculum is applied to experimental host plant in pots. These pots are maintained for 14 days after which they are harvested, the root length measured and then stained. The resulting entry points are counted to ascertain the infectivity potential.

(A) Equipments and Reagents

a. Pots (5 x 7 cm in size)
b. Sorghum seeds (*Sorghum vulgare*)
c. Scissors and needles
d. Petri dish (grided)
e. Water bath
f. Glass slides and cover slips
g. Compound microscope
h. Coarse sieve to prevent root loss during washing/changing solutions
i. Plastic vials with tight-sealing lids for storage of stained samples in 50% lycerol
j. Potassium hydroxide solution (5-10%)
k. Alkaline H_2O_2 (25% Ammonia solution: 3 ml + 10% H_2O_2: 30 ml + Distilled water 67 ml)
l. 1% HCl
m. 50% glycerol-water (v/v) solution for de-staining and storage of stained roots.
n. Lactoglycerol (Lactic acid: 876 ml + Glycerine: 64 ml + Distilled water: 60 ml)

(B) Staining solutions

a. 0.01 % acid fuschin: 0.01 g acid fuschin in 100ml acetoglycerol.
b. 0.05% trypan blue: 0.05 g trypan blue in 100ml acetoglycerol.
c. 0.03% Chlorozol black E (CBE) in lactoglycerol (1:1:1 lactic acid, glycerol and water).
d. Dissolve CBE in water before adding equal volumes of lactic acid and glycerol.

(C) Procedure

a. Place 100 g test sample in a pot
b. Dilute the inoculum with sterilized sand if the inoculum is very rich
c. Plant 10-12 pre germinated seeds of Sorghum and grow for 14 days
d. Harvest the pots and recover roots (fine roots can be rescued using sieve) completely.
e. Chop the roots equally 1 cm in length.
f. Measure and record the root length (using grid line intersect method described below) from each sample/dilution.
g. Clear the roots in KOH solution and stain the root pieces (described below)
h. Count the number of infection points/entry points formed on randomly picked 100 segments
i. Calculate the average number of entry points formed in 1 cm segment
j. Calculate the total number of infection points/infective propagules (IP) by multiplying the average number of entry points formed in 1 cm segment by the total root length.
k. Extrapolate the IP present as numbers per gram of substrate/inoculum

(D) Estimation of root length (Tennant D 1975)

(1) **Equipment**

a. Scissors
b. Petri dish (9 cm in size consisting 1.33 cm x 1.33 cm grids)
c. Wash bottle
d. Stereo zoom microscope

(2) The lines intersect (Tennant 1975) method is used to estimate the length of hyphae and roots. Root length is measured by dispersing roots against a grid of squares on the bottom of a tray. The roots are spread apart from one another over a grid in 2 mm to 10-mm depth of water. The eyes of the observer are cast along all the horizontal and vertical lines of the grid and root is counted using a hand held click counter.

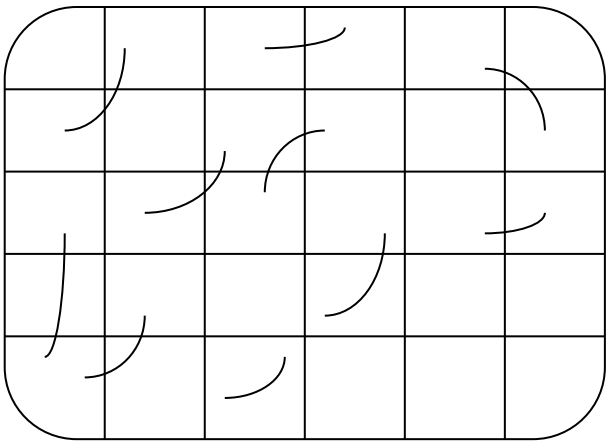
The root length is calculated as follows:

Root length = No. of intersects x 11/14 x grid size

Where, 11/14 is a constant, and the size of the grid is the length of one side of one square of the grid.

Counting root intersections

a. Randomly disperse root in dish with grid lines.
b. Count the intersects on roots across the horizontal and vertical lines.
c. An example of 10 root segments is presented to show how the root length is calculated



Horizontal intersects (HI)
2

2

2

2
(Total 8 HI)

Vertical intersects (VI) (Total 7 VI)

2 1 2 0 2

Total number of intersects = HI+VI = 8 + 7 (example) = 15

Thus, the root length = 11/14 X 15 X 1 (as the grid size is 1cm) = 11 cm (example)

(3) **Clearing and staining root specimens**

Clearing and staining procedures requires root samples that should be washed free of soil. It is important that KOH and staining solution volumes are sufficient for the amount of roots being processed and that, roots are not tightly clumped together for uniform contact with solutions. To ensure uniform staining, the roots should be chopped in to smaller (1-2 cm) segments.

(a) Wash root specimens under running tap water thoroughly. Place them in beaker containing 5-10% KOH solution for about 15-30 minutes. The concentration of KOH and time of incubation of roots depend upon the age and tenderness of the roots.

(b) Pour off the KOH solution and rinse the roots well in a beaker using at least three complete changes of tapwater or until no brown colour appears in the rinse water.

(c) Cover the roots with alkaline H_2O_2 at room temperature for 10 minutes or until roots are bleached.

(d) Rinse the roots thoroughly using at least three complete changes of tap water to remove the H_2O_2.

(e) Cover the roots with 1% HCl and soak for 3-4 min. And then pour off the solution. DO NOT rinse after this step because the specimens must be acidified for proper staining.

(f) Incubate the roots with staining solution (0.01% acid fuchsine in lactoglycerol or 0.05% trypan blue in lacto phenol) and keep them overnight for staining.

(g) Place the root specimens in glass petriplate /multiwell plate for destaining. The destaining solution (50% glycerol) is the standard used in step 4, but of course, without the stain.

(4) **Sample storage and slide preparation**

If clearing and staining is not possible immediately then fresh roots can be kept moist and stored at 5 °C (for several days), or may be preserved in 50% ethanol for months together in tightly sealed vials.

Staining quality is subsequently improved by destaining roots in 50% glycerol for several months prior to observation to allow excess stain to leach from roots. Semi-permanent slides of stained roots can be made with PVLG mountant. For temporary slide the stained roots can be observed in plain lactoglycerol.

1F. METHOD OF ANALYSIS FOR POTASH SOLUBILISING BIOFERTILIZERS (KSB)

1. Estimation of total viable count and contamination

1. Apparatus-

1.1 Pipettes graduated 1ml and 10 ml
1.2 Dilution bottles or flasks
1.3 Petri dishes clear, uniform, flat-bottomed
1.4 Hot-air oven : Capable of giving uniform and adequate temperature, equipped with a thermometer, caliberated to read upto 250^0C and with venus suitably located to assure prompt and uniform heating.
1.5 Autoclave
1.6 Incubator
1.7 Handy tally or mechanical counting device
1.8 Ph meter

2. Reagents

2.1 Medium

Use plating medium of the following composition for total viable count and contamination

Medium for analysis of total viable count and contamination (ingredients g/lit)

Manitol	15.0
Yeast extract	3.0
Peptone	2.0
Agar	18.5
Trace element solution	1 ml
Distilled Water	1000 ml

Trace element solution(ingredients g/lit)

Sodium molybdate	0.20
Boric acid	0.28
Manganese sulphate	0.23
Copper sulphate	0.01
Zinc sulphate	0.03
Distilled Water	1000 ml

Medium for studying zone of solubilization in KSB(Ingredients g/lit)

Glucose	5.0
Magnesium sulphate	0.005
Ferric chloride	0.1
Calcium carbonate	2.0
Pottassium mineral (mica powder)	2.0
Calcium phosphate	2.0
Distilled Water	1000

2.2 Sterilizing and preparation procedure for plates

2.2.1 Sterilize the sampling and plating equipment with dry heat in a hot air oven at less than 160^0C for not less than 2 hours;

2.2.2 Sterilize the media by autoclaving at 120^0C for 20 min. To permit passage of steam into and from cloased containers when autoclaved, keep stoppers slightly lossened or plugged with cotton. Air from within the chamber of the sterilizer should be ejected allowing steam pressure to rise.

2.3 Preparation of plating medium and pouring

2.3.1 Prepare growth medium in accordance with the composition of the specific biofertiliser.

2.3.2 Melt the required amount of medium in boiling water or by exposure to flowing steam in partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Melt enough medium which will be used within 3 h. Re-sterilization of the medium may cause partial precipitation of ingredients.

2.3.3 When holding time is less than 30 min. promptly cool the molten medium to about 45^0C, and store until used, in a water bath or incubator at 43 to 45^0C. Introduce 12 to 15 ml of liquefied medium or appropriate quantity depending on size of the Petri dish at 42 to 44^0C into each plate. Gently lift the cover of the dish just enough to pour in the medium. Sterilizes the lips of the medium containers by exposure to flame.

(a) Immediately before pouring.
(b) Periodically during pouring, and
(c) When pouring is complete for each batch of plates, if portions of molten medium remain in containers and are to be used without subsequent sterilization for pouring additional plate. As each plate is poured thoroughly mix the medium with test portions in the petri dish.

2.3.4 By rotating and tilting the dish and without splashing the medium over edge spread the medium evenly over the bottom of the plate. Provide conditions so that the medium solidifies with reasonable promptness (5-10 min) before removing the plates from level surface.

3. Preparation of Serial Dilution for Plate Counts

3.1 Dispense 10 g of inoculants to 90 ml of sterile distilled dematerialized water and shake for 10 min on a reciprocal shaker or homogenizer. Make serial dilutions upto 10^{10}. Take 1:0 ml or suitable aliquots of 10^6 to 10^9 dilutions using sterile pipettes and deliver to petri dishes containing set medium as given in 2.1 and spread it uniformly with a spreader or use droplet method. Invert the plates and promptly place them in the incubator.

4. Incubation of Plates

4.1 Label the plates and incubate at 28± 2^0C for 4 to 6 days.

4.2 Colony counting aids
Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in centimeter square. Record the total number of colonies with the hand tally. To distinguish colonies from dirt, specks and other foreign matter, examine doubtful objects carefully.

4.3 Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies which absorb congo red and stand out as reddish colonies. *Fraturia aurentia* (KMB) stand out as white-opaque glistening and domed colonies. Count such colony numbers and calculate figures in terms of per litre, of carrier. Also check for freedom from contamination at 10^5 dilution.

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

5 Method of estimation of K solubiliation zones

5.1 Take 10 g of KSB in 90 ml sterile distilled water

5.2 Make a tenfold dilution series upto 10^7

5.3 Take 1.0 ml aliquot of 10^5 to 10^7 dilutions using sterile pipettes and deliver to petri dishes containing K-solubilization zone media.

5.4 Spread it uniformly, invert the plates and incubate for up to 2 weeks at 28±2^0C.

5.5 Count the colonies showing solubilization zones and measure the diameter of solubilization zone. Calculate average zone of solubilization in mm.

1G METHOD OF ANALYSIS OF FOR ZINC SOLUBILIZING BIOFERTILIZERS

1. Estimation of total viable count and contamination

2. Apparatus

2.1 Pipettes graduated 1ml and 10 ml
2.2 Dilution bottles or flasks
2.3 Petri dishes clear, uniform, flat-bottomed
2.4 Hot-air oven : Capable of giving uniform and adequate temperature, equipped with a thermometer, calibrated to read upto 250^0C and with venus suitably located to assure prompt and uniform heating.
2.5 Autoclave
2.6 Incubator
2.7 Hand tally or mechanical counting device
2.8 pH meter

3. Reagents

3.1 Medium

Use plating medium of the following compostion for total viable count and contamination

Medium for analysis of Total Viable Count, Contamination and zone of solubilization for Zn solubilisation for Zn solublizing biofertilizer

(Ingredients g/lit)

Glucose	10.0
Zinc oxide	1.0
Amm sulphate	0.5
Potassium chloride	0.2
Yeast extract	0.5
Ferrous sulphate	0.01
Manganese sulphate	0.01
Di Pot Hyd. Phosphate	0.5
Distilled Water	1000 ml

3.2 Sterilizing and preparation of plates

3.2.1 Sterilize the sampling and plating equipment with dry heat in a hot air oven at less than 160^0C for not less than 2 hours;

3.2.2 Sterilize the media by autoclaving at 120^0C for 20 min. To permit passage of steam into and from closed containers when autoclaves, keep stoppers slightly lossened or plugged with cotton. Air from within the chamber of the sterilizer should be ejected allowing steam pressure to rise.

3.3 Preparation of plating medium and pouring

3.3.1 Prepare growth medium in accordance with the composition of the specific biofertilizer.

3.3.2 Melt the required amount of medium in boiling water or by exposure to flowing steam in partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Melt enough medium which will be used within 3 hours. Re-sterilization of the medium may cause partial precipitation of ingredients.

3.3.3 When holding time is less than 30 min. promptly cool the molten medium to about 45^0C, and store until used, in a water bath or incubator at 43- to 45^0C. Introduce 12 to 15 ml of liquefied medium or appropriate quantity

depending on size of the petri dish at 42 to 44^{0}C into each plate. Gently lift the cover of the dish just enough to pour in the medium. Sterilize the lips of the medium containers by exposure to flame.

(a) Immediately before pouring.
(b) Periodically during pouring, and
(c) When pouring is complete for each batch of plates, if portions of molten medium remain in containers and are to be used without subsequent sterilization for pouring additional plate. As each plate is poured thoroughly mix the medium with test portions in the petridish.

3.3.4 By rotating and tilting the dish and without splashing the medium over edge spread the medium evenly over the bottom of the plate. Provide conditions so that the medium solidifies with reasonable promptness (5-10 min) before removing the plates from level surface.

4. Preparation of Serial Dilution for Plate Counts

4.1 Dispense 10 g of inoculants to 90 ml of sterile distilled dematerialized water and shake for 10 min on a reciprocal shaker or homogenizer. Make serial dilutions upto 10^{10}. Take 1:0 ml or suitable aliquots of 10^6 to 10^9 dilutions using sterile pipettes and deliver to petri dishes containing set medium as given in 2.1 and spread it uniformly with a spreader or use droplet method. Invert the plates and promptly place them in the incubator.

5. Incubation of Plates

5.1 Label the plates and incubate at 28± 2^{0}C for 4 to 6 days.

5.2 Colony counting aids : Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in centimeter square. Record the total number of colonies with the hand tally.

5.3 Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies which absorb congo red and stand out as reddish colonies. Zinc solubilizing biofertilisers stand out as white, translucent, glistening and elevated colonies. Count such colony numbers and calculate figures in terms of per litre, of carrier. Also check for freedom from contamination at 10^5 dilution.

5. **Counting**

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

6. Method of estimation of Zinc solubiliation zones

6.1 Take 10 g of ZSB in 90 ml sterile distilled water
6.2 Make a tenfold dilution series upto 10^7
6.3 Take 1.0 ml aliquot of 10^5 to 10^7 dilutions using sterile pipettes and deliver to petri dishes containing Zinc solubilization zone media.
6.4 Spread it uniformly, invert the plates and incubate for up to 2 weeks at 28±2^{0}C.
6.5 Count the colonies showing solubilization zones and measure the diameter of solubilization zone. Calculate average zone of solubilization in mm.

1.H. METHOD OF ANALYSIS OF ACETOBACTER (spp.)

1. **Apparatus** - Same as Rhizobium
2. **Reagents -**

2.1 Medium

Use plating medium of the following composition for viable count and contamination :-

Medium for analysis of total viable count and contamination (ingredients gram/litre)

Sucrose	100 g
K_2HPO_4 (Di-Potassium Hydrogen Phosphate	0.4 g
KH_2PO_4 (Potassium di-hydrogen phosphate)	0.6 g
$MgSO_4$ (Magnesium Sulphate)	0.2 g
Calcium Chloride	0.02 g
Sodium Molybdate	0.02 g
Ferric Chloride	0.01 g
Bromothymol blue solution (0.5% in 0.2 m KOH)	5.0 ml
Distilled water	1000ml
pH	5.5 g
agar agar	18.5 g

3. Sterilizing & preparation procedure for plates:

Same as Rhizobium

3.1Preparation of Plating Medium and Pouring

Same as Rhizobium

4. **Preparation of Serial Dilution for Plate Counts**:

Same as Rhizobium.

5. **Incubation of Plates**:

Same as Rhizobium.

5.1. Colony counting Aids : Count the colonies with the aid of magnifying lens under uniform and properely controlled, artificial illumination. Use a colony counter, equipped with guide plate and rules in centimeter square. Record the total number of cononies with hand tally. Avoid mistaking particles of undissolved medium or precipitated matter in plates for pin point colonies. To distinguish colonies from dirt, specks and foreign matter, examine doubtful objects carefully.

5.2 Count all plates but consider for the purpose of calculation only those plates showing more than 30 and less than 300 colonies per plate. Acetobacter a nitrogen fixing bacteria stand out as irregular 2-3 mm diameter, smooth flat with bright yellow or yellow with oran e centre colour. Count such colony numbers and calculate figures in terms of per litre, of carrier. Also check freedom from contamination at 10^5.

6. Test for confirmation:

6.1 Apparatus

Same as Azospirillum.

6.2 Reagents

6.2.1 Medium : Semi solid for pellicle formation (ingredients gm per liter)

Sucrose	100 g
K_2HPO_4 (Di-Potassium Hydrogen Phosphate	0.4 g

KH_2PO_4 (Potassium di-hydrogen phosphate)	0.6 g
$MgSO_4$ (Magnesium Sulphate)	0.2 g
Calcium Chloride	0.02 g
Sodium Molybdate	0.02 g
Ferric Chloride	0.01 g
Bromothymol blue solution (0.5% in 0.2 m KOH)	5.0 ml
Distilled water	1000ml
pH	5.5 g
agar agar	1.75 g

1.I. Methods of Analysis of Phosphate Solubilizing Fungal Blofertilizer

1. Apparatus required:

1.1. Pipettes, graduated, 1 ml and 10 ml

1.2 Conical Flasks, 150 ml and 250 ml

1.3 Screw-Capped Tubes, 10 ml

1.4 Incubator 1.5 Petri Dishes

1.5 Hot Air Oven

1.6 pH Meter

1.7 Autoclave

1.8 Haemocystometer

1.9 Compound Microscope

1.10 Glass Slides and Cover Slips

1.11 Forceps

1.12 Needles

1.13 Glass Rods

2. Preparation of serial dilution for plate count (spore / cfu)

2.1 Dispense 30 g of PSFI to 270 ml of sterile distilled/ demineralized water and shake for 10 min on a reciprocal shaker or homogenizer. Make serial dilutions up to 10-7 by suspending 10 ml aliquot of previous dilution to 90ml of water. Take 0.1 ml or suitable aliquotes upto 10-7dilutions using sterile pipettes and deliver to Petri dishes containing set medium as given in section 1.2 and spread it uniformly with a spreader. Invert the plates and promptly place them in the incubator.

2.2 Incubation of plates

2.2.1 Label the plates and incubate at 28+ 1o C for 2 to 4 days.

2.2.2. Colony counting aids Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in Centimeter Square. Record the total number of colonies with the hand tally. Avoid mistaking particles of un dissolved medium or precipitated matter, in plates for pinpoint colonies. To distinguish colonies from dirt, specks and other foreign matter, examine doubtful objects carefully.

2.2.3. Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Count such colony numbers and calculate figures in terms of per gram/litre of carrier. Also check for freedom from contamination at 10-0 dilution

2.2.4 Counting

2.2.4.1 Count the colonies showing hallow Zones and measure their diameter.

3. Determination of soluble phosphorus using ascorbic acid method:

3.1 Apparatus Spectrometer capable of transmission measurement at 840 to 880 mm.
Extractant: It is Olsen extract.

3.2 Reagents
Ammonium Molybdate [(NH4)6 Mo7O24.4H2O]
L- Ascorbic Acid
p-Nitro phenol
4NH2SO4

3.3 Preparation of reagents

3.3.1 Sulphomolybdic Acid:-
3.3.1.1- Take 20 g of ammonium molybdate and dissolve in 300 ml of distilled water.
3.3.1.2- Add slowly 450 ml of 10N - H2SO4.
3.3.1.3- Cool the above mixture and add 100 ml of 0.5 percent solution of antimony Potassium tartrate.
3.3.1.4- Cool and make the volume to one liter. Store in glass bottle away from direct sunlight.

3.3.2 Preparation of Mixed Reagent
Add 1.5 g of L-ascorbic acid in 100ml of the above stock solution and mix. Add 5ml of this solution to develop colour. Mixed reagent is to be prepared fresh as it does not keep for more than 24 h.

3.3 Procedure
(i) Preparation of Sample
Prepare broth medium in 100 ml aliquots in 6 no., 250 ml conical flasks and sterilize in autoclave at 1210C for 20 min.
(ii) Inoculation of Medium
Select one PSFI colony of the type that has been counted as PSFI (showing sufficient zone of solubilization) and streak on set medium in a Petri dish. Use this pure culture for inoculating the broth. Inoculate 3 flasks and keep 3 flasks as uninoculated control. Incubate the flasks over rotary shaker for 12 days at 28$\pm$1°C.
After 12 days, filter the contents of each flask separately through Whatman No. 42 filter paper or centrifuge at 10,000 rpm for 15 min.
(iii) Add 10 ml of filtrate/ centrifugate to 50 ml of olsen extractant and shake for 30 min over rotary shaker.
(iv) Filter the suspension through Whatman filter paper No. 40. If the filtrate is coloured then add a tea spoon of Dacro-60 (activated phosphorous free carbon), reshake and filter.
(v) Take a known aliquot (5 to 25 ml) of the extract in a 50 ml volumetric flask.
(vi) Add 5 drops of p-nitrophenol indicator (1.5 per cent solution in water) and adjust the pH of the extract between 2 and 3 with the help of 4N H2SO4 . The yellow colour will disappear when the pH of the solution becomes 3. Swirl gently to avoid loss of the solution along with the evolution of CO2.
(vii) When the CO2 evolution has subsided, wash down the neck of the flask and dilute the solution to about 40 ml.
(viii) Add 5 ml of the sulphomolybdic acid mixed reagent containing ascorbic acid, swirl the content and make up the volume.
(ix) Measure the transmission after 30 min at 880 nm using red filter. The blue colour developed remains stable upto 60 minutes.
(x) Record the concentration of phosphorous (P) in the extract form the standard curve and calculate the concentration of soluble phosphorous as follows:"

3.4 Calculations

(a) Weight of the substance taken = x g

(b) Volume of the extract added = 50 ml

(c) Volume of the extract taken for P determination = y ml

(d) Volume made after colour developed = 50 ml

(e) Reading from the standard curve against percent transmission recorded = z ppm

(f) Soluble Phosphorous percent $p = \frac{Z \times 50 \times 10^{-6} \times 50 \times 100}{Y \times X}$

3.5 Preparation of standard Curve

Prepare standard curve using 0.1 to 0.6 ppm P in 50 ml volumetric flask. Plot the standard curve by taking concentration of soluble P on x- axis and percent T on y- axis using a semi log graph paper. It is a straight line relationship between the soluble P and percent T when plotted on a semi-log graph paper."

3. Sterilization and preparation of MPN tubes

(Same as specified in the Method Analysis of Azospirillum at serial number 1C)

4. Preparation of serial dilution for MPN count

(Same as specified in the Method Analysis of Azospirillum at serial number 1C)

5. Incubation of tubes

(Same as specified in the Method Analysis of Azospirillum at serial number 1C)

6. Counting-Yellowish pellicle formation below 1mm of upper surface of nitrogen free semi solid media. Counting the tubes or plates which have turned yellowish in colour after inoculation and ascertained the presence of pellicle in undisturbed medium. To determine usual contamination on the same examine doubtful objects carefully.

7. Method for estimating MPN Count

Count all tubes which have turned yellowish and consider them for the purpose of calculation. Count such type of tubes and tally this count with MPN table (as specified in the Method of Analysis of Azospirillum at serial number 1C in Table 1) to get the number of cells per gram of carrier or number of cells per ml of liquid.

1.J. METHOD OF ANALYSIS OF CARRIER BASED CONSORTIA OF BIOFERTILISER AND LIQUID CONSORTIA OF BIOFERTILISER

1. Methods of Analysis of Rhizobium Biofertiliser – Same as specified for Rhizobium at 1.A.
2. Methods of Analysis of Azotobacter – same as specified for Azotobacter at 1.B.
3. Methods of Analysis Azospirillum – same as specified for Azospirillum as 1.C.
4. Methods of Analysis of Phosphate Solubilising Bacteria PSB – same as specified for Phosphate Solubilising Bacteria at 1.D.
5. Methods of Analysis of Potash Mobilising Bacteria (KMB) – same as specified for Phosphate Solubilising Bacteria at 1.F.

MAINTENANCE AND PREPARATION OF CULTURE AND QUALITY CONTROL AT BROTH STAGE

RHIZOBIUM:

1. Maintenance of pure cultures

Maintain pure culture of rhizobia on yeast extract mannitol agar (YEMA) slants to the following composition.

Mannitol	10.0g
Potassium hydrogen phosphate (K_2HPO_4)	0.5 g
Magnesium sulphate ($MgSO_4$ 7 H_2O)	0.2g
Sodium chloride (NaCl)	0.1 g
Calcium carbonate ($CaCO_3$)	1.0 g
Yeast extracts	1.0g
Agar	18g
Distilled water	1 liter
pH	6.8- 7.0

Transfer a loopful of the pure culture to each of the agar slant aseptically in an inoculation room and incubate at 28+/- 2^o C for 3 to 10 days depending upon the species of Rhizobium. Always keep culture at 4^o C.

2. Preparation of Inoculums Cultures

2.1 Prepare yeast extract mannitol broth of the composition as given in1.1.minus the agar.

2.2 Transfer a loop full of the culture.

3. Quality Control Test Recommended at Broth Stage:

3.1 Qualitative Tests

3.1.1 Check for freedom from the visible contaminants

3.1.2 The pH of the bacterial broth shall normally be between 6.5 and7.5.

3.1.3 Smear and Gram stain

3.1.3.1 Reagents

a. Ammonium oxalate crystal violet stain- weigh 0.2 g of crystal violet and dissolve in 20 ml of 95 percent ethyl alcohol. Dissolve separately 0.8 g of ammonium oxalate in 30 ml of distilled water. Mix the two solution and filter through a filter paper.

b. Iodine solution

Iodine	1.00 g
Potassium Iodide	2.00 g
Distilled water	300 ml

Weigh the ingredient and dissolve in water. Filter through a filter paper.

c. Erythrosine

Erythrosine	1.00 g
Phenol	5.00 g
Distilled water	100 ml

Weigh the ingredient, dissolve in distilled water and filter through a filter paper.

3.1.3.2 Procedure

Prepare a smear on a clean microscope slide, fix over a flame by gently and intermittent heating, air cool and flood with ammonium, oxalate crystals violet stain for 1 min. After removing the excess of ammonium oxalate crystals violet,

wash the slide under a gentle stream of running tap water. Flood the slide with iodine solution for half a minute remove excess stain wash 95 percent ethyl alcohol and finally wash under a gentle stream of running tap water. Flood the slide with erythrosine stain for about 3min, wash under a gentle stream of running tap water and dry between the folds of a filter paper. Examine the slide under a compound microscope using an oil immersion objective.

Note:- A smear prepared from undiluted broths should be free from Gram positive cells. The presence of a few gram positive cells in occasional fields which may be due to dead cells in the medium may be disregarded.

3.1.4 Absence of Growth on Glucose- Peptone Agar

The composition of the glucose –peptone agar is as follows:

Glucose	10.0 g
Peptone	20.0 g
Sodium chloride (NaCl)	5.0 g
Agar (IS6850)	15.0 g
Distilled water	100 ml
Bromocresol purple alcoholic solution (1.6%)	10 ml
pH	7.2

Note:- when a loopful of the broth is streaked in to this Medium and incubated at 28+/- 2^{o} C for 24 h, the purple-violet colour of the medium(due to the indicator bromo-cresol purple) shall Not change . If the colour changes to yellow (acidic reaction) or blue (alkaline reaction) the broth is grossly contaminated. Hence the broth should be rejected.

3.1.5. Streak on yeast extract mannitol agar with congo-red

When a loopful of broth culture is streaked to a plate of this medium and incubated at 28± 2^{o} C for 3 - 10 days, it shows colonies of bacteria with growth characteristics same as that of the pure culture used in the preparation of the broth, otherwise the broth should be rejected.

3.2 Quantitative Test

3.2.1 Viable or plate counts - Serially dilute one milliliter of the broth to obtain dilutions of the order of 10^{6} to $10^{9.}$ Plate 0.2 ml aliquots of the dilution on YEMA plates and incubate at 28±2^{o}C for 2 to 6 days, depending on the species of Rhizobium. The counts of viable Rhizobium in the final broth from shake culture or fermenters shall be not less than 10^{8} to 10^{9} cells/ ml. Otherwise, the broth should be rejected.

AZOSPIRILLUM

1. Maintenance of pure cultures.

Maintain pure culture of Azospirillum on nitrogen free bromothymol blue medium and maintain as solid medium.

Transfer a loopful of pure culture to each of the agar culture tube aseptically in an inoculation room and incubate 37$\pm$ 2^{o}C for three days and keep in undisturbed. Always keep pure culture below 5^{o} C.

2. Preparation of Inoculums culture and Mass culture:

Inoculums culture and mass culture of this standard shall be prepared as described for Rhizobium of this standard.

3. Quality Control Test Recommended at Broth Stage

3.1. **Quality Test**

3.1.1 Check for free from contaminants by preparing slide and observing under microscope.

3.1.2 The pH of bacterial broth shall normally be between 7.0 to 8.0.

3.1.3 Gram staining test shall be carried out as descried for Rhizobium of this standard.

3.1.4. See the colour change in the media after 24 hours from inoculation. The colour will change from green to blue.

3.1.5 Watch the pellicle just below the surface of the media. It is checked on the third day after keeping inoculated broth undisturbed.

3.2 **Quantitative Test**

3.2.1 Most probable Number (MPN) as given in Annexure E. The counts of Azospirillum in the final broth from shake culture or fermenter shall be not less than 10^8 to 10^9 cells/ ml. Otherwise the broth should be rejected.

AZOTOBACTOR

1. Maintenance of pure cultures.

Maintain pure culture of Azotobacter on slants of the following composition

Agar	20 g
Sucrose	20 g
Ferrous Sulphate	0.1 g
Dibasic Potassium Phosphate	1.0 g
Magnesium Sulphate	0.5 gm
Calcium carbonates	2.0gm
Sodium Molybdate	0.005gm

Transfer a loopful pure culture to each of agar slants aseptically in an inoculation room and incubate at 28±2° C for 3 to 10 days depending up on the species of Azotobactor. Always keep pure culture at 5° C.

2. Preparation of inoculums culture

2.1 Prepare Jensen's media broth of the compositor as given in 1.1 minus the agar.

2.2 Transfer a loop full of the culture into a 100 ml/250 ml conical flask containing the broth. Incubate the flasks at 28±2° C on a rotary shaker for 2 to 6 days.

3 Quality control Tests recommended at Broth stage.

3.1 Quality test

3.1.1. Check for free from contaminants by preparing slide and observing under microscope.

3.1.2 The pH by bacterial broth shall normally be between 6.5 to 7.0

3.1.3 Gram staining test shall be carried out as described for Rhizobium of this standard.

3.2 Quantitative test

3.2.1 Viable cell count same as Rhizobium

4. Packing, Marking, Storage and Use

4.1 Packing - Biofertilizers shall be packed in suitable plastic bags/packets, thickness of which shall not be less than 75-100 micron or in suitable plastic bottles.

4.2 Marking - Each polyethylene pack shall be marked legibly and indelibly with the following information:

(a) Name of the product,
(b) Name and address of the manufacturer,
(c) Crops for which intended;
(d) Type of the carrier used;
(e) Batch number;
(f) Date of manufacture;
(g) Expiry date which shall not be less than 6 months from the date of manufacture in case of carrier based powdered/granulated formulation of Rhizobium, Azotobacter, Azospirillum and PSB biofertilisers and liquid based Rhizobium biofertiliser, while it shall not be less than 12 months from the date of manufacture in case of liquid based Azotobacter, Azospirillum and PSB biofertilisers;
(h) Net mass in kg/gram and area meant for;
(i) Storage instruction worded as under;
"STORE IN COOL PLACE AWAY FROM DIRECT SUN LIGHT AND HEAT"
(j) Any other information required under the standards of weights and Measure (Packaged Commodities) Rule.1977.

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

4.4 Direction for use of biofertiliser shall be printed briefly on the packets as given below.

"The contents of the packet are sufficient enough for seed treatment on to the given area to be broadcasted or given seedling 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 surface gunny bag and sow them immediately.

Use only for the crops mentioned. Use before the expiry date and do not expose to direct sunlight or heat.

Biofertiliser is not a chemical fertilizer hence do not mix inoculated seeds or inoculants with agro-chemicals."

4.5 Storage

Inoculants shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at temperature of 20^{o}C. It shall also be the duty of the manufacturer to instruct the retailers and in turn, the users about the precaution to be taken during storage.

Schedule –IV
[See clause 2(h) and (q)]
Part – A
Specifications of Organic Fertilizers

1. City compost:

(i)	Moisture, per cent by weight	15.0-25.0
(ii)	Colour	Dark brown to black
(iii)	Odour	Absence of foul odour
(iv)	Particle size	Minimum 90% material should pass through 4.0 mm IS sieve
(v)	Bulk density (g/cm^3)	<1.0
(vi)	Total organic carbon, per cent by weight, minimum	12.0
(vii)	Total Nitrogen (as N), per cent by weight, minimum	0.8
(viii)	Total Phosphates (as P_2O_5), per cent by weight, minimum	0.4
(ix)	Total Potash (as K_2O), per cent by weight, minimum	0.4
(x)	C:N ratio	<20
(xi)	pH	6.5 - 7.5
(xii)	Conductivity (as dsm^{-1}), not more than	4.0
(xiii)	Pathogens	Nil
(xiv)	Heavy metal content, (as mg/Kg), maximum	
	Arsenic as (As_2O_3) Cadmium (as Cd) Chromium (as Cr) Copper (as Cu) Mercury (as Hg) Nickel (as Ni) Lead (as Pb) Zinc (as Zn)	10.00 5.00 50.00 300.00 0.15 50.00 100.00 1000.00

2. Vermicompost

(i)	Moisture, per cent by weight	15.0-25.0
(ii)	Colour	Dark brown to black
(iii)	Odour	Absence of foul odour
(iv)	Particle size	Minimum 90% material should pass through 4.0 mm IS sieve
(v)	Bulk density ($g/cm^{3)}$	0.7 -0.9
(vi)	Total organic carbon, per cent by weight, minimum	18.0
(vii)	Total Nitrogen (as N), per cent by weight, minimum	1.0
(viii)	Total Phosphate (as P_2O_5), per cent by weight, minimum	0.8
(ix)	Total Potassium (as K_2O), per cent by weight, minimum	0.8
(x)	Heavy metal content, (as mg/Kg), maximum	
	Cadmium (as Cd) Chromium (as Cr) Nickel (as Ni) Lead (as Pb)	5.0 50.00 50.00 100.00

3. Phosphate Rich Organic Manure (PROM)

(i)	Moisture per cent by weight, maximum	15.0-25.0
(ii)	Particle size-minimum 90% material should pass through 4.0 mm IS sieve	
(iii)	Bulk density (g/cm^2)	1.646
(iv)	Total organic carbon per cent by weight, minimum	7.87
(v)	Total nitrogen (as N) per cent by weight, minimum	0.42
(vi)	Total phosphates (as P_2O_5) per cent by weight, minimum	10.42
(vii)	Total potash (as K_2O) per cent by weight, minimum	-
(viii)	C:N ratio	18:73:1
(ix)	pH(1:5 solution) maximum	6.72
(x)	Conductivity (as dSm^{-1}) not more than	8.27
(xi)	Heavy metal content (as mg/kg), maximum	
	Arsenic (as As_2O_3)	10.0
	Cadmium (as Cd)	5.0
	Chromium (as Cr)	50.0
	Copper (as Cu)	300.0
	Mercury (as Hg)	0.15
	Nickel (as Ni)	50.0
	Lead (as Pb)	100.0
	Zinc (as Zn)	1000.0

4. Organic Manure

(i)	Moisture per cent by weight, maximum	25.0
(ii)	Particle size	Minimum 90% material should pass through 4.0 mm IS sieve
(iii)	Bulk density (g/cm^2)	<1.0
(iv)	Total organic carbon per cent by weight, minimum	14.0
(v)	Total nitrogen (as N) per cent by weight, minimum	0.5
(vi)	Total phosphates (as P_2O_5) per cent by weight, minimum	0.5
(vii)	Total potash (as K_2O) per cent by weight, minimum	0.5
(viii)	NPK nutrients – Total N, P_2O_5 and K_2O nutrient should not be less than 3%	
(ix)	C:N ratio	<20
(x)	pH	6.5-7.5
(xi)	Conductivity (as dSm^{-1}) not more than	4.0
(xii)	Pathogen	Nil
(xiii)	Heavy metal content, (as mg./kg), maximum	
	Arsenic (as As_2O_3)	10.0
	Cadmium (as Cd)	5.0
	Chromium (as Cr)	50.0
	Copper (as Cu)	300.0
	Mercury (as Hg)	0.15
	Nickel (as Ni)	50.0
	Zinc (as Zn)	1000.0

Note : The source of organic manure is any of the plant biomass/ animal biomass/ animal Excreta)

5. Bio-enriched Organic Manure

1.	Moisture percent by weight, maximum	30-40
2.	Particle size	Minimum 90% material should pass through 4.0 mm IS sieve
3.	Bulk density (g/cm3)	< 1.0
4.	Total Viable count (N, P, K and Zn Bacteria) or (N and P bacteria) or (N and K Bacteria)	5.0 x10^6 (within 3 months from the date of manufacture)
5.	Total organic carbon, per cent by weight, minimum	14.0
6.	Total Nitrogen (as N) per cent by weight, minimum	0.8
7.	Total Phosphates (as P_2O_5) per cent. by weight minimum	0.5
8.	Total Potash (as K_2O) per cent by weight, minimum	0.8
9.	NPK nutrients - Total of N, P_2O_5 and K_2O nutrient should not be less than 3%	
10.	C:N Ratio	<18
11.	pH	6.5-8.0
12.	Conductivity (as dSm^{-1}) not more than	4.0
13.	Heavy metal content (as mg/kg), maximum • Arsenic (as As_2O_3) • Cadmium (as Cd) • Chromium (as Cr) • Copper (as Cu) • Mercury (as Hg) • Nickel (as Ni) • Lead (as Pb) • Zinc (as Zn)	 • 10.00 • 5.00 • 50.00 • 300.00 • 0.15 • 50.00 • 100.00 • 1000.00

6. Bone meal , raw

(i) Moisture per cent by weight, maximum	8.0
(ii) Acid insoluble matter per cent by weight, maximum	12.0
(iii) Total phosphorous (as P_2O_5) per cent by weight, minimum	20.0
(iv) 2 per cent citric acid soluble phosphorous (as P_2O_5) per cent by weight , minimum	8.0
(v) Nitrogen content of water insoluble portion per cent by weight ,minimum	3.0
(vi) Particle size –the material shall pass wholly through 2.36 mm IS sieve of which not more than 30 per cent shall be retained on 0.85 mm IS sieve.	

7. Bone meal, Steamed

(i) Moisture per cent by weight, maximum.	7.0
(ii) Total phosphorous (as P_2 O_5) per cent by weight, minimum	22.0
(iii) 2 percent citric acid soluble phosphorous (as P_2O_5) per cent by weight ,minimum	16.0
(iv)Particle size- Not less than 90% of the material shall pass through 1.18 mm IS sieve	

8 Potash derived from Rhodophytes

(i)	Moisture percent by weight, maximum	5.0
(ii)	Water soluble Potash, percent by weight, minimum	20.0
(iii)	Total Sulphur (as S), percent, by weight, minimum	1.5
(iv)	Heavy metal content (as mg/kg), maximum	
	Arsenic as (As_2O_3)	10.0
	Cadmium (as Cd)	5.0
	Chromium (as Cr)	50.0
	Copper (as Cu)	300.00
	Mercury (as Hg)	0.15
	Nickel (as Ni)	50.00
	Lead (as Pb)	100.00
	Zinc (as Zn)	1000.00

Part –B

TOLERANCE LIMIT OF ORGANIC FERTILISER

A sum total of nitrogen, phosphorus and potassium nutrients shall not be less than 1.5% in City Compost and shall be not less than 2.5% in case of vermin-compost.

Part- C

PROCEDURE FOR DRAWL OF SAMPLE OF ORGANIC FERTILISER

(As per methodology as mentioned under schedule-ii, part-A of FCO,1985)
The Inspector shall draw any sample of Organic fertilizer in accordance with the procedure of drawl mentioned under schedule-ii, Part-A.

1. General requirements of sampling

In drawing samples, the following measures and precautions should be observed:

(a) Samples shall not be taken at a place exposed to rain /sun.
(b) The sampling instruments shall be clean and dry when used.
(c) The material being sampled, the sampling instruments and the bags of samples should be free from any adventitious contaminations.
(d) To draw a representative sample, the contents of each bag selected for sampling should be mixed as thoroughly as possible by suitable means.
(e) The sample should be kept in suitable, clean dry and airtight glass or screwed hard polythene bottle of about 400gm capacity or in a thick-gauged polythene bag. This should be put in a cloth bag, which may be sealed with the Inspector's seal after putting inside the detailed description as specified in Form 'P'. Identifiable details may also be put on the cloth bag like sample No./Code No. or any other details which enables its identification.
(f) Each sample bag should be sealed air tight after filling and marked with details of sample and type of fertilizer and the name of Inspector who has collected sample.

2 Sampling from bagged material

(i) Scale of sampling

(a) **Lot (for manufacturers/importers) -** All bags in a single consignment of the material of the same grade and type drawn from a single batch of the manufacturer/importer shall constitute a lot. If a consignment is declared to consist of different batches of manufacturer/import, all the bags of each batch shall constitute a separate lot. In the case of a consignment drawn from a continuous process, 2000 bags (or 100 tonnes) of the material shall constitute a lot.

(b) **Lot (for dealer)** - The lot is an identifiable quantity of same grade and type of fertilizer stored at an identifiable place subject to a maximum limit of 100 tones. The inspector based on visible appearance of bags, their packing and storage conditions shall identify the lot. The stock of less than 100 tones with a dealer may also constitute one or more lots, if the material (fertiliser) of different sources and brand is available in such quantities.

(c) **Selection of bags for sampling –**

(i) The number of bags to be chosen from a lot shall depend upon the size of the lot as given below:

Lot size (No. of bags) (N)	No. of bags to be selected for sampling(n)
Upto 10	1
11-100	2
101-200	3
201-400	4
401-600	5
601-800	6
801-1000	7
1001-1300	8
1301-1600	9
1601-2000	10

All the bags of a lot should be arranged in a systematic manner. Start counting from any bag randomly; go on counting as 1,2,3,……..up to R and so on. R being equal to the integral of N/n. Thus every 4^{th} bag counted shall be withdrawn and all bags shall constitute the sample bags from where the sample is to be drawn for preparing a composite sample.

(ii) **Sampling from big godowns/high stackings** - If the procedure given in Para 2 (i)(c) is not possible to be adopted, the sample should be drawn from the randomly selected fertilizer bags from different layers, from top and from all open sides in a zig zag fashion.

(iii) **Sampling from small godowns** - All the fertilizer bags of the same grade and type of each manufacturer though received on different dates shall be segregated and properly stacked. All bags of same grade and type of fertilizer manufactured by a particular manufacturing unit may be considered as one lot based on their physical conditions and the sample shall be drawn as per procedure laid down in para 2 (i) (c) and 4.

(iv) **Sampling from damaged stocks**

(a)In case of torn or lumpy bags, damaged fertilizer bags or sweepings, the stock should be arranged according to identifiable lots. From each lot the number of bags shall be selected as per procedure 2 (i) (c). If the bags allow the use of sampling probe conveniently, the samples should be drawn from sampling probe.

(b) In case it is not possible to use the sampling probe, the bags may be opened and fertilizer material mixed together uniformly by hammering the big lumps or putting pressure, if required, and then samples drawn by using suitable sample device.

3. Sampling probe

(i) An appropriate sampling instrument to be used by the Inspector for collection of a representative sample is called sampling probe. The probe may comprise of a slotted single tube with solid cone tip made of stainless steel or brass. The length of the probe may be approximately 60 to 65 cms and the diameter of the tube may be approximately 1.5 cm and the slot width may be 1.2 to 1.3 cms. The probe may be used if the physical condition of the fertilizers and the packing material permits its use.

(ii) In case of high-density polythene packing and also when the fertilizer material is not in free flowing condition, the use of sampling probe may not be possible. In such case, selected bags for drawing samples may be opened and the fertilizers may be taken out of the bags and spread on a clean surface and samples drawn with the help of a suitable sampling device, which may be made of stainless steel or brass cup.

4. Drawal of samples from bags

(i) Drawal of sample and preparation of composite samples.
Draw, with an appropriate sampling instrument, (sampling probe) small portions of the material from the selected bags as per procedure in para 2(i) (b), 2(ii), 2(iii) and 2(iv) (a). The sampling probe shall be inserted in the bag from one corner to another diagonally and when filled with fertilizer, the probe is withdrawn and fertilizer is emptied in a container/ or on polythene sheet/ or on a clean hard surface and made into one composite sample.

(ii) If the bags do not permit the use of sampling probe, empty the contents of the bags on a level, clean and hard surface and draw a composite sample by the process of quartering as described under para 3(ii) or 5.

4A. Weight of one Sample

One sample of organic fertilizer shall have approximate 400gms weight.

5. Preparation of composite sample

If the composite sample collected from the different selected bags is larger than required weight, its size shall be reduced by method of quartering as detailed below:-

Spread the composite sample on a level, clean, hard surface, flatten it out and divide it into four equal parts. Remove any diagonally opposite parts. Mix the two remaining parts together to form a cone, flatten out the cone and repeat the operation of quartering till a composite sample of required weight is obtained.

6. Preparation of test sample and reference sample

(i) The composite sample obtained above shall be spread out on a clean, hard surface and divided into three approximately equal portions each of the weight as specified in Para 4A. Each of these samples shall constitute the test sample.

(ii) Each test sample shall be immediately transferred to a suitable container as defined under para 1(e). The slip with detailed description may be put inside the sample bag. Each bag shall also be properly labeled as mentioned in para 1(f).

(iii) Each test sample container shall then be sealed with seals of the inspector, if possible, seal of the manufacturer/ importer/ dealer or purchaser as the case may be also be affixed.

(iv) Out of the three samples collected, one sample so sealed shall be sent to the Incharge of the laboratory notified by the state Government under clause 29 or National/Regional Centre of Organic Farming, Ghaziabad,

Bangalore, Bhubaneshwar, Hisar, Imphal, Jabalpur or Nagpur for analysis. Another sample shall be given to the manufacturer or importer or dealer or the purchaser, as the case may be. The third sample shall be sent by the Inspector to his next higher authority for keeping in safe custody. Any of the latter two samples may be sent for referee analysis as provided for under sub-clause (2) of clause 29B.

Part- D

METHODS OF ANALYSIS OF ORGANIC FERTILISERS

1. Estimation of pH

- Make 25 g of compost into a suspension in 50 ml of distilled water and shake on a rotary shaker for 2 hours.
- Filter through Whatman No. 1 or equivalent filter paper under vacuum using a Buchner funnel.
- Determine pH of the filtrate by pH meter.

2. **Estimation of moisture**

Weigh to the nearest mg about 5 gm of the prepared sample in a weighed clean, dry Petri Dish. Heat in an oven for about 5 hours at 65^{o}C ±1^{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

3. **Estimation of Bulk density**

Requirements

-100 ml Measuring cylinder, Weighing balance
-Rubber pad [1 sq foot; 1 inch thickness], Hot air oven

Method

- Weigh a dry 100ml cylinder (W1gm)
- Cylinder is filled with the sample up to the 100 ml mark. Note the volume (V1 ml)
- Weigh the cylinder along with the sample (W2 gm)
- Tap the cylinder for two minutes.
- Measure the compact volume (V2 ml)

Calculation

$$\text{Bulk density} = \frac{\text{Weight of the sample taken}(W2-W1)}{\text{Volume } (V1-V2)}$$

4. Estimation of conductivity

Requirements

- 250 ml flask, Funnel [OD-75mm]

- 100ml Beaker, Analytic Balance
- Potassium Chloride [AR grade] , Filter paper
- Conductivity meter [With temperature compensation system]

Method

- Pass fresh sample of organic fertilizer through a 2-4mm sieve.
- Take 20 gm of the sample and add 100 ml of distilled water to it to give a ratio of 1:5
- Stir for about an hour at regular intervals.
- Calibrate the conductivity meter by using 0.01 M potassium chloride solution.
- Measure the conductivity of the unfiltered organic fertilizer suspension.

Calculation

Express the results as millimho's or dsm^{-1} at 25°C specifying the dilution of the organic fertilizer suspension viz, 1:5 organic fertilizer suspensions.

5. Estimation of Organic Carbon

Apparatus

(i) Silica/Platinum crucible 25 g cap.
(ii) Muffle Furnace

Procedure

Accurately weigh 10 gm of sample dried in oven at 105°C for 6 hrs, in a pre weighed crucible and ignite the material in a Muffle furnace at 650 – 700°C for 6-8 hrs. Cool to room temperature and keep in Desiccator for 12 hrs. Weigh the contents with crucible

Calculation

Calculate the total organic carbon by the following formulae:-

$$\text{Total Organic matter \%} = \frac{\text{Initial wt – final wt}}{\text{wt. of sample taken}} \times 100$$

.

$$\text{Total C\%} = \frac{\text{Total organic matter \%}}{1.724}$$

6. Estimation of total Nitrogen

As mentioned under Schedule – II, Part-B, 3 (v) of FCO,1985.

Apparatus

1. Suitable Kjeldahl assembly consisting of 500-800 ml round bottom, digestion flask and Kjeldahl distillation assembly consisting of 500-800 ml distillation flask, splash head tube and condenser, all with appropriate glass joints. The length of the condenser's delivery tube should be long enough to keep immersed in a flask for ammonia absorption.
2. Kjeldahl digestion unit with heating control, suitable for 500-800 ml flasks.

Reagents

a. Sulphuric acid – 93-98% H_2SO_4, N-free
b. Salicylic acid, reagent grade, N-free
c. Sodium thiosulphate ($Na_2S_2O_3$ $5H_2O$), reagent grade

d. Zinc dust- impalpable powder
e. Copper sulphate
f. Potassium or sodium sulphate
g. 45% NaOH solution. Dissolve 450 gm of Sodium hydroxide pellets in distilled water and make up the volume to 1000ml
h. Methyl red indicator – Dissolve 1gm methyl red in 200 ml alcohol
i. Hydrochloric or sulphuric acid standard solution – 0.1N or as per requirement
j. Sodium hydroxide standard solution 0.1N or as per requirement.

Procedure

1. Place weighed sample (0.7-2.2gm) in digestion flask.
2. Add 40 ml H_2SO_4 containing 2 grams salicylic acid. Shake until thoroughly mixed and let stand, with occasional shaking, 30 minutes or more.
3. Then add (i) 5 grams $Na_2S_2O_3.5H_2O$ or (ii) 2 grams zinc dust (as impalpable powder not granulated zinc or filing).
4. Shake the flask and let it stand for five minutes then heat over low flame until frothing ceases.
5. Turn off heat, add 0.7 grams copper sulphate, 15 gm powdered K_2SO_4 (or anhydrous Na_2SO_4), and boil briskly until solution clears, continue boiling for another at least 2 hours.
6. Remove from burner and cool, add 200 ml of water and swirl the flask to dissolve all the contents.
7. Transfer to 500 ml volumetric flask, giving several washings with water to the digestion flask. Make up the volume to 500 ml.
8. Take 25 ml aliquot in the distillation flask, add 300 ml water and a pinch of zinc dust
9. Take 20 ml of standard acid solution in the receiving conical flask, add 4 drops of methyl red indicator and keep the flask at the lower end of the condenser in such a way that the lower tip of the condenser is fully immersed in acid solution.
10. Add 30 ml of 45% NaOH to the distillation flask, gently so that the contents do not mix.
11. Immediately connect the flask to distillation assembly and swirl to mix the contents. Heat until all the ammonia is distilled (at least 150 ml distillate).
12. Remove from receiving flask. Rinse outlet tube into receiving flask with a small amount of distilled water.
13. Titrate the contents in the receiver conical flask with standard NaOH solution.
14. Determine blank on reagents using same quantity of standard acid in receiving conical flask.

Calculation

$$\text{Nitrogen \% by weight} = \frac{1.401(V_1N_1-V_2N_2)-(V_3N_1-V_4N_2) \times df}{W}$$

where

V_1 = Volume in ml of standard acid taken in receiver flask for sample
V_2 = Volume in ml of standard NaOH used in titrating standard acid in receiver flask after distillation of test sample
V_3 = Volume in ml of standard acid taken in receiver flask for blank
V_4 = Volume in ml of standard NaOH used in titrating standard acid in receiver flask after distillation in blank
N_1 = Normality of standard acid
N_2 = Normality of standard NaOH
W = Weight in gm of sample taken
df = Dilution factor of sample

7. Estimation of C: N Ratio

Method

Calculate the C:N ratio by dividing the organic carbon value with thetotal nitrogen value.

8. Estimation of phosphate

Preparation of sample - Accurately weigh 10 gm oven dried sample in 50 g cap. silica crucible and ignite it to 650^{o} – 700^{o}C for 6-8 hrs toobtain ash. Cool and keep in a Dessicator.Transfer the contents to a 100 ml beaker. Add 30 ml 25% HCl.Washthe crucible with 10 ml 25% HCl twice and transfer the contents toBeaker. Heat over hot plate for 10-15 min. Keep for 4 hrs. Filterthrough Whatman No.1 filter paper. Wash with distilled water 4-5times (till acid free).Make up the volume of filtrate to 250 ml in a volumetric flask. Estimate total P by gravimetric quinoline molybdate method asdescribed under Schedule – II, Part B, 4(ii) of FCO 1985.

Gravimetric quinoline molybdate method for determination of total phosphorus

Reagents

1. **Citric molybdic acid reagent** – Dissolve 54 gm, 100% molybdic anhydride $(Mo)_3$) and 12 gm NaOH with stirring in 400 ml hot water and cool. Dissolve 60 gm citric acid in mixture of 140 ml HCl and 300 ml water and cool. Gradually add molybdic solution to citric acid solution with stirring. Cool, filter and dilute to 1 lit. (solution may be green or blue colour depending on exposure to light) If necessary add 0.5% $KBrO_3$ solutiondrop by drop until green colour becomes pale. Store in dark in polyethylene bottle.
2. **Quinoline solution** – Dissolve 50 ml synthetic quinoline with stirring in mixture of 60 ml HCl and 300 ml water. Cool dilute to 1 lit and filter. Store in polyethylene bottle.
3. **Quimociac reagent** – Dissolve 70 gm of sodium molybdate dehydrate in 150 ml water. Dissolve 60 gm citric acid in mixture of 85 ml HNO_3 and 150 ml water and cool. Gradually add molybdate solution to citric acid-nitric acid mixture with stirring. Dissolve 5 ml synthetic quinoline in mixture of 35 ml HNO_3 and 100 ml water. Gradually add this solution to molybdate –citric-nitric acid solution mix and let it stand for 24 hr. Filter, add 280 ml acetone, dilute to 1 lit with water and mix well. Store in polyethylene bottle.

Procedure

1. Digest 1 gm sample as described above and dilute to 200 ml.
2. In 500 ml Erlenmeyer flask pipette aliquot containing not more than 25mg P_2O_5 dilute to approximately 100 ml with water. Proceed with one of the following method.

    a. Add 30 ml citric-molybdic acid reagent and boil gently for 3 min (solution must be precipitate free at this stage). Remove from heat and swirl carefully. Immediately add from burette 10 ml quinoline solution with continuous swirling (add first 3-4 ml drop wise and remainder in steady stream) or
    b. Add 50 ml quimociac reagent, cover with watch glass place on hot plate in well ventilated hood and boil for 1 min.

After treatment with a or b cool to room temperature, swirl carefully 3-4 time during cooling, filter through sintered glass Gooch crucible Grade 4 (30 ml capacity), previously dried at 250^{o}C and weighed, and wash 5 times with 25 ml portion of water. Dry crucible and contents for 30 min. at 250^{o}C. Cool in

dessiccator to constant weight as $(C_9H_7N)_3H_3PO_4.12MoO_3$. Subtract blank weight. Multiply by 0.03207 to obtain weight of P_2O_5. Report as percent P_2O_5.

9. Estimation of Potassium

Flame photometry method:- Total Potassium are usually determined by dry ashing at 650-700 Degree Centigrade and dissolving inconcentrated hydrochloric acid.

Reagent and Standard curve

(1) Potassium chloride standard solution: Make a stock solution of 1000 ppm K by dissolving 1.909 g. of AR grade potassiumchloride (dried at 60°C for 1 h) in distilled water 1 ; anddiluting up to 1 litre. Prepare 100 ppm standard by diluting 100ml of 1000 ppm stock solution to 1 litre with extractingsolution.

(2) Standard curve: Pipette 0,5, 10,15 and 20 ml of 100 ppmsolution into 100 ml volumetric flasks and make up the volumeupto the mark. The solution contain 0,5, 15 & 20 ppm Krespectively.

Procedure:

- Take 5g sample in a porcelain crucible and ignite the material to ash at650-700 C in a muffle furnace.
- Cool it and dissolve in 5 ml concentrated hydrochloric acid, transferin a 250 ml beaker with several washing of distilled water and heat it.
- Again transfer it to a 100 ml volumetric flask and make up the volume.
- Filter the solution and dilute the filtrate with distilled water so that theconcentration of K in the working solution remains in the range of 0to20 ppm, if required.
- Determine K by flame photometer using the K- filter after necessarysetting and calibration of the instrument.
- Read similarly the different concentration of K of the standardsolution in flame photometer and prepare the standard curve by plottingthe reading against the different concentration of the K.
- Calculation: Potash (K) %by weight = R X 20 X diluting factor, whereR= ppm of K in the sample solution (obtained by extra plotting from standard curve).

10. Estimation of Cadmium, Copper, Chromium, Lead, Nickeland Zinc

Material Required

1. Triacid mixture: Mix 10 parts of HNO_3(Nitric acid), 1 part of H_2SO_4 (Sulphuric Acid) and 4 parts of $HClO_3$ (Perchloric Acid)
2. Conical flask, 250ml
3. Hot plate
4. Whatman filter paper No.42
5. Atomic Absorption Spectrophotometer (AAS)

Processing of sample

Take 5.0 g or suitable quantity of oven dried (105^0C) sample thoroughly ground and sieved through 0.2 mm sieve in a conicalflask.Add 30 ml triacid mixture,cover it with a small glass funnel forrefluxing. Digest the sample at 200^0C on a hot plate till the volumeis significantly reduced with a whitish residue.After cooling, filter the sample with Whatman No. 42 filter paper andmake up to 100 ml in a volumetric flask.

Preparation of working standards

Cadmium - As mentioned under Schedule – II, Part B, 8(x) of FCO (1985)

Copper - As mentioned under Schedule – II, Part B, 8(iv) of FCO(1985)

Chromium - Dilute 1, 2, 3 and 4 ml of standard 100 ppm Chromium standard solution with doubled distilled water involumetric flasks and make up the volume to 100 ml to obtainstandards having concentrations of 1, 2, 3, 4 ppm

Lead - As mentioned under Schedule – II, Part B, 8(v) of FCO (1985)

Nickel - Dilute 1,2,3 and 4 ml of standard 100 ppm Nickelstandard solution with doubled distilled water in volumetric flasksand make up the volume to 100ml to obtain standards havingconcentrations of 1, 2, 3, 4 ppm

Zinc - As mentioned under Schedule – II, Part B, 8(ii) of FCO (1985)

(Alternatively dilute 1, 2, 3 and 4 ml of 100ppm standard stock solution of respective element with doubled distilled water in volumetric flasks and make up the volume to 100ml to obtain standards having concentrations of 1, 2, 3, 4 ppm)

Measurement of Result

Estimate the metal concentrations of Cd, Cu, Cr, Pb, Ni, Zn by flaming the standard solution and samples using atomic absorptionspectrophotometer (AAS) as per the method given for instrument atrecommended wavelength for each element. Run a blank followingthe same procedure.

Expression of Result

Express the metal concentration as mg/kg on oven dry weight basis in 3 decimal units. (Reference: Manual for Analysis of Municipal Solid Waste (compost): Central Pollution Control Board)."

11. Estimation of Mercury

Reagents:

(a) Concentrated Nitric acid (HNO_3)
(b) Concentrated Sulphuric acid (H_2SO_4)
(c) Potassium persulphate (5% solution): Dissolve 50g of $K_2S_2O_8$ in1 litre of distilled water.
(d) Potassium permagnate (5% solution): Dissolve 50g of $KMnO_4$in 1 litre of distilled water.
(e) Hydroxylamine sodium chloride solution: Dissolve 120 g ofHydroxyl amine salt and 120 g of sodium chloride (NaCI) in 1litre distilled water.
(f) Stannous chloride (20%): Dissolve 20 g of $SnCI_2$ in 100 mldistilled water.

Materials required

(a) Water bath
(b) Flameless atomic absorption spectrophotometer or cold vapour mercury analyzer.
(c) BOD bottle , 300 ml

Processing of sample:

(a) Take 5 g (finely ground but not dried) sample in an oven at a temperature of 105^0C for 8 hours for moisture estimation.
(b) Take another 5 g sample (finaly ground but not dried) in a BOD bottle, add to it 2.5 ml of conc. HNO_3, 5ml of cone. $H_2SO_4$and 15 ml of 5% $KMnO_4$.
(c) After 15 minutes add 8 ml of 5% $K_2S_2O_8$.
(d) Close the bottle with the lid and digest it on a water bath at 95^0C for 2 hours.
(e) After cooling to room temperature add 5 ml hydroxylaminesodium chloride soln.

Measurement:

Reduction of the digested sample is brought out with 5 ml of 20% SnC12 immediately before taking the reading, using a cold vapourmercury analyzer.

Expression of results:
Express the mercury concentration as mg/Kg on oven dry weight basis in 3 decimal units.
(Reference: Manual for Analysis of Municipal Solid Waste (compost); Central Pollution Control Board).

12. Estimation of Arsenic
Processing of sample – Suspend 10 gm finely ground sample in 30 ml aquaregia (HNO_3 + HCl in a ratio of 1:3) in a beaker. Keep on hot platetill moist black residue is obtained (do not dry). Add 5 ml aquaregiaand allow to dry on a hot plate till residue is moist. Dissolve the residuein 30 ml conc. HCl and filter through Whatman No.1 filter paper in 100ml volumetric flask. Wash filter paper 3-4 times with double distilledwater. Make up the volume to 100 ml. Take 1 ml of this solution in 100ml volumetric flask, add 5ml conc. HCl and 2 gm KI and make up thevolume to 100 ml.Prepare standards having concentration of 0.05, 0.1 and 0.2 ppm by diluting 0.05, 0.1 and 0.2 ml, respectively of standard Arsenic solutionwith double distilled water in volumetric flask and make up the volumeto 100 ml

Measurement – Estimate Arsenic using vapour generation assemblyattached to Atomic Absorption Spectrophotometer as per the proceduregiven for the instrument.

13. Pathogenicity Test
Apparatus
1. Samples of Compost
2. Lactose Broth of Single and Double Strength
3. Culture Tubes
4. Durham Tubes
5. Bunsen Burner
6. Sterile Pipettes
7. Incubator, Autoclaves,
8. Petri-Plates
9. Inoculation Loops

Preparation of Culture Media
A. For Presumptive Test
1. Lactose Broth

Beef Extract :	6.0 g
Peptone :	10.0 g
Lactose :	10.0 g
D.W. :	1000 ml

B. For Confirmative Test
1. Eosine Methylene Blue Agar Media (EMB Media)

Peptone :	10.0 g
Lactose :	5.0 g
Sucrose :	5.0 g
K_2HPO_4 :	2.0 g
Eosine Y :	0.4 g
Methylene Blue :	0.06 g
Agar :	15.0 g
D.W. :	1000 ml

C. For Completed Test

1. Nutrient Agar

Beef Extract : 3.0 g
Peptone : 5.0 g

Procedures

A. Presumptive Test

1. Prepare 12 tubes of lactose broth for each sample and close the tube with cotton plugs/caps and autoclave at 121^{o}C for 20 min.
2. Fill Durham tubes with sterilized distilled water and keep in beaker and autoclave at 121^{o}C for 20 min.
3. Suspend 30 g of compost sample in 270 ml of sterile distilled water and serially dilute upto 10^{-4} dilution as per Schedule III, Part D,serial number 3 of FCO (1985)
4. Suspend 1 ml suspension from 10^{-1} to 10^{-4} in 3 tubes for each dilution
5. Insert distilled water filled Durham tube in inverted position in eachtube and close the tube again
6. Inoculate tubes at 36^{o}C for 24h in incubator

Result

- Production of gas within 24h - Confirms the presence of coliforms in the sample
- Production of gas within 48h - Doubtful Test.
- No Gas Production - Negative Test

B. Confirmative Test

Confirmative test is for differentiating the coliforms from non-coliforms as well as Gram negative and Gram positive bacteria. In thistest, the EMB agar plates are inoculated with sample from positivetubes producing gas. Emergence of small colonies with dark centresconfirms the presence of Gram negative, lactose fermenting coliformbacteria. Sometimes some of the non-coliforms also produce gas,therefore, this test is necessary.

1. Prepare EMB agar plates with the composition as per the method given above
2. Inoculate plates with the help of inoculation loop with streaking of samples showing positive/doubtful tests in the presumptive test
3. Incubate plates at 30± 1^{o}C for 12 h in incubator
4. Dark centred or nucleated colonies appear which may differentiate between *E. coli* and *E. aerogenes* based on size of colonies andmetallic sheen

Result

E. coli colonies on this medium are small with metallic sheen, where as*E. aerogenes* colonies are usually large and lack the sheen.

C. Completed Test

This test is required for further confirmation.

Procedure

1. Pick up a single colony from EMB agar plate
2. Inoculate it into lactose broth and streak on a nutrient agar slant
3. Incubate the slants
4. Perform Gram reaction after attaining the growth

Result

Gram-negative nature of bacteria is indicative of a positive completed test.”

Schedule V

[see clause 2(h) and (q)]

Part – A
Specifications of Non-edible De-oiled cake fertilizers

1. General Specification of Non-edible De-oiled cake

Sl.No.	Parameter	Requirement
i	Moisture percent by weight maximum	10.00
ii	Ash content % by weight (maximum)	15.0
iii	Total Carbon, % by weight, minimum	23.0
iv	Total Nitrogen (as N) % by weight, Minimum	1.5
v	Total Phosphates (as P_2O_5) % by weight Minimum	0.20
vi	Total Potash (as K_2O) % by weight, minimum	0.50
vii	pH	4.5-6.5
viii	Conductivity	<4.0

Part – B

TOLERANCE LIMIT OF NON-EDIBLE DE-OILED CAKE FERTILIZERS

0.5 Unit for Nitrogen, Phosphorous and Potassium Nutrients combined.

Part- C

PROCEDURE FOR DRAWL OF SAMPLE OF NON-EDIBLE DE-OILED CAKE FERTILIZERS

The Inspector shall draw any sample of Non-edible De-oiled cake fertilizer in accordance with the procedure of drawl mentioned under schedule-II, part-A of FCO, 1985.

Part- D

METHODS OF ANALYSIS FOR NON-EDIBLE DE-OILED CAKE FERTILIZERS

1. Estimation of pH

As mentioned in Schedule IV Part D (1) of FCO 1985

2. Estimation of moisture

As mentioned in Schedule IV Part D (2) of FCO 1985

3. Estimation of ash Content

Apparatus

i) Silica/Platinum crucible 25 g cap.
ii) Muffle Furnace
iii) Desiccator

Weigh to the nearest mg about 5 gm of oven dried powdered sample in a weighed clean, dry Petri Dish. Incinerate in a muffle furnace for about 6-8 hours at 650-700°C to constant weight. Cool in a dessicator and weigh. Report percentage of ash content obtained.

Calculation:

$$\text{Ash content in percent by weight} = \frac{100x(C-A)}{B-A}$$

A = Weight of the empty crucible
B = Weight of the empty crucible plus material before ashing
C = Weight of the empty crucible plus material after ashing

4 Estimation of conductivity

As mentioned in Schedule IV Part D (4) of FCO 1985

5. Estimation of organic carbon

As mentioned in Schedule IV Part D (5) of FCO 1985

6. Estimation of total nitrogen

Apparatus

1. Suitable Kjeldahl assembly consisting of 500-800 ml round bottom, digestion flask and Kjeldahl distillation assembly consisting of 500-800 ml distillation flask, splash head tube and condenser, all with appropriate glass joints. The length of the condenser's delivery tube should be long enough to keep immersed in a flask for ammonia absorption.
2. Kjeldahl digestion unit with heating control, suitable for 500-800 ml flasks.

Reagents

a. Sulphuric acid – 93-98% H_2SO_4, N-free
b. Salicylic acid, reagent grade, N-free
c. Sodium thiosulphate ($Na_2S_2O_3$ $5H_2O$), reagent grade
d. Zinc dust- impalpable powder
e. Copper sulphate
f. Potassium or sodium sulphate
g. Selanium powder
h. Red Mercury oxide (HgO)
i. 45% NaOH solution. Dissolve 450 gm of Sodium hydroxide pellets in distilled water and make up the volume to 1000ml
j. Methyl red indicator – Dissolve 1gm methyl red in 200 ml alcohol
k. Hydrochloric or sulphuric acid standard solution – 0.1N or as per requirement
l. Sodium hydroxide standard solution 0.1N or as er requirement.

Procedure

1. Place weighed finely powdered sample (0.5-1.0 gm) in digestion flask.
15. Add 1 gm digestion mixture (20 g $CuSO_4$+ 3 gm selenium dust + 1 gm HgO)
16. Add 50 ml conc H_2SO_4
17. Shake the flask and let it stand for five minutes then heat over low flame until frothing ceases.
18. Turn off heat, add 15 - 20 gm powdered K_2SO_4 (or anhydrous Na_2SO_4), and boil briskly until solution clears,
19. Add 5 gm Sodium thiosulphate continue boiling for another at least 2-3 hours.
20. Remove from burner and cool, add 200 ml of water and swirl the flask to dissolve all the contents.
21. Transfer to 500 ml volumetric flask, giving several washings with water to the digestion flask. Make up the volume to 500 ml.
22. Take 25 ml aliquot in the distillation flask, add 300 ml water and a pinch of zinc dust
23. Take 20 ml of standard acid solution (N/10 H_2SO_4) in the receiving conical flask, add 4 drops of methyl red indicator and keep the flask at the lower end of the condenser in such a way that the lower tip of the condenser is fully immersed in acid solution.
24. Add 40 ml of 45% NaOH to the distillation flask, gently so that the contents do not mix.
25. Immediately connect the flask to distillation assembly and swirl to mix the contents. Heat until all the ammonia is distilled (at least 150 ml distillate).

26. Remove from receiving flask. Rinse outlet tube into receiving flask with a small amount of distilled water.
27. Titrate the contents in the receiver conical flask with standard NaOH solution.
28. Determine blank on reagents using same quantity of standard acid in receiving conical flask.

Calculation

$$\text{Nitrogen \% by weight} = \frac{1.401(V_1N_1-V_2N_2)-(V_3N_1-V_4N_2) \times df}{W}$$

where
V_1 = Volume in ml of standard acid taken in receiver flask for sample
V_2 = Volume in ml of standard NaOH used in titrating standard acid in receiver flask after distillation of test sample
V_3 = Volume in ml of standard acid taken in receiver flask for blank
V_4 = Volume in ml of standard NaOH used in titrating standard acid in receiver flask after distillation in blank
N_1 = Normality of standard acid
N_2 = Normality of standard NaOH
W = Weight in gm of sample taken
df = Dilution factor of sample

7. Estimation of C:N ratio

Method

Calculate the C:N ratio by dividing the organic carbon value with the total nitrogen value

8. Estimation of Phosphate

As mentioned in Schedule IV Part D (8) of FCO 1985

9. Estimation of Potassium

As mentioned in Schedule IV Part D (9) of FCO 1985

Appendix

Emblem
Form K-1
[see Clause 30]

MEMORANDUM TO ACCOMPANY ORGANIC FERTILIZER/ BIOFERTILIZER/ NON-EDIBLE DE-OILED CAKE FERTILIZER SAMPLE FOR ANALYSIS

No
From

_____
_____

To
Incharge
Organic Fertilizer/ Biofertilizer/ Non-edible de-oiled cake fertilizer Quality Control Laboratory

_____

The Organic Fertilizer/ Biofertilizer/ Non-edible de-oiled cake fertilizer sample as per the details given below are sent for analysis:-

1. Name of Organic Fertilizer/ Biofertilizer/ Non-edible de-oiled cake fertilizer
2. Date of sampling
3. Physical condition
4. Code number of sample

The analysis report may be forwarded to

_____
_____

Place

Date

Signature and metallic seal
Impression of Fertilizer Inspector

Emblem
Form L-1
[see Clause 30]
ANALYSIS REPORT OF ORGANIC FERTILIZER SAMPLE

No._____

Government of _____
_____
_____
(Name of the Laboratory)

Date _____

To
The Fertiliser Inspector
_____
_____

The analysis report of the organic fertilizer sample forwarded vide your reference No._____ dated _____ is as per details given below :

(1) Name of organic fertilizer _____
(2) Date of sampling _____
(3) Code No. of sample as indicated by the Inspector _____
(4) Date of receipt of the sample in laboratory_____
(5) Laboratory sample No. _____
(6) Date of analysis of sample _____
(7) Analysis of organic fertilizer (on fresh weight basis)

Sl. No.	Specification as per FCO	Composition as per analysis	Variation	Permissible tolerance limit
1	2	3	4	5

(A) Physical Characterstics –
- i. Moisture content _____
- ii. Bulk density _____
- iii. Particle size _____

(B) Chemical Characteristics –
- i. Total Organic Carbon _____
- ii. Total Nitrogen _____
- iii. C:N _____
- iv. Phosphorus _____
- v. Potassium _____
- vi. pH _____
- vii. Conductivity _____

(C) Heavy Metal
- i. Cadmium _____

ii. Chromium _____
iii. Copper _____
iv. Mercury _____
v. Nickel _____
vi. Lead _____
vii. Zinc _____

Remarks : The sample is/is not according to specification and fails in _____
_____
_____

Signature of the Incharge
(Testing Laboratory)

Copy to :
Director of Agriculture
_____
_____

Emblem
Form L-2
[see Clause 30]
ANALYSIS REPORT OF BIOFERTILISER SAMPLE

No._____ Date _____

Government of _____
_____
(Name of the Laboratory)

To
The Fertiliser Inspector
_____

The analysis report of the biofertiliser sample forwarded vide your reference No._____ dated _____ is as per details given below :

(1) Name of biofertilizer _____
(2) Date of sampling _____
(3) Code No. of sample as indicated by the Inspector _____
(4) Date of receipt of the sample in laboratory _____
(5) Laboratory sample No. _____
(6) Date of analysis of sample _____
(7) Analysis of Biofertiliser (on fresh weight basis)

Sl. No.	Specification as per FCO (Rhizobium, Azotobacter, Azospirillum, PSM)	Composition as per analysis (Rhizobium, Azotobacter, Azospirillum, PSM)	Variation	Permissible tolerance limit
1	2	3	4	5

(A) Physical Characterstics –
  i. Moisture content _____
  ii. Particle size _____
(B) Chemical Characteristics –
  i. pH _____
(C) Microbial Characteristics
  i. Viable Cell Count _____
  ii. ContaminationLevel _____
(D) Efficiency Characteristics
  *(i) Nodulation Test
  **(ii) Nitrogen fixed (mg)/g of sucrose consumed
  ***(iii) Formation of white pellicle in semi solid Nitrogen free bromothymol blue media
  +(iv) (a) Solubilization zone (mm)
  (b) P-phosphorus (%) Spectrophotometer

Remarks : The sample is/is not according to specification and fails in _____
_____

Signature of the Incharge
(Testing Laboratory)

Copy to :
Director of Agriculture

Emblem
Form L-3
[see Clause 30]
ANALYSIS REPORT OF NON-EDIBLE DE-OILED CAKE FERTILISER SAMPLE

No._____ Date _____

Government of _____
_____
_____
(Name of the Laboratory)

To
The Fertiliser Inspector
_____
_____

The analysis report of the organic fertilizer sample forwarded vide your reference No._____ dated _____ is as per details given below :

(1) Name of Non-edible de-oiled cake fertiliser _____
(2) Date of sampling _____
(3) Code No. of sample as indicated by the Inspector _____
(4) Date of receipt of the sample in laboratory _____
(5) Laboratory sample No. _____
(6) Date of analysis of sample _____
(7) Analysis of Non-edible de-oiled cake fertilizer (on fresh weight basis)

Sl. No.	Specification as per FCO	Composition as per analysis	Variation	Permissible tolerance limit
1	2	3	4	5

(A) Physical Characterstics –
i. Moisture content _____
ii. Particle size _____

(B) Chemical Characteristics –
i. Total Organic Carbon _____
ii. Total Nitrogen _____
iii. C:N _____
iv. Phosphorus _____
v. Potassium _____
vi. pH _____
vii. Conductivity _____
viii. Total Ash _____
ix. Others _____

Remarks : The sample is/is not according to specification and fails in _____

Signature of the Incharge
(Testing Laboratory)

Copy to :
Director of Agriculture
_____

Minimum Laboratory Requirements for Quality Testing Laboratory for Biofertilizer and Organic Fertilizers

(S.O. 2724E) Gazette Notification Dated 8th November 2010)

In pursuance of sub-clause (2) of clause 29 of the Fertiliser (Control) Order, 1985, the Controller with a view to ensure accurate analysis of fertilizer samples, hereby specifies that every laboratory notified for testing the samples of biofertilizer and organic fertilizer under sub-clause (1) shall possess within one year from the date of publication of this notification, the following minimum laboratory equipment and other laboratory facilities, namely:-

(A) Equipment and other facilities for testing samples of biofertilizers;

1.	**Hot air oven** (up to 250^{0}C)	Chamber size minimum 24" x 24" x 24"
2.	**Vertical Autoclave**	16" Dia x 24"height)
3.	**BOD incubator**	capacity min-9cft., 5^{0}C- 50^{0}C
4.	**pH Meter**	
5.	**Laminar Air Flow**	minimum size 3 x 2 x 2ft.
6.	**Reciprocal/Orbital incubator shaker**	
7.	Colony counter or Mechanical counting device	
8.	Serological water bath	
9.	Electronic balance	
10.	Water distillation unit	
11.	Kjeldahl digestion unit	
12.	Kjeldahl distillation assembly	
13.	Standard IS sieve	
14.	Spectrophotometer	360-960nm
15.	Binocular research microscope	40 and 100X objective, preferably with phase contrast attachment having 10X, 40X and 100 X phase objectives
16.	Flame photometer	
17.	Rotary shaker	
18.	Vacuum filtration device	
19.	Vacuum pump	
20.	Earthen Pots	
21.	Cooling storage cabinet for samples	10-25^{o}C
22.	Rotary vacuum dryer	

(B) Equipments and other facilities for testing samples of organic fertilizer laboratories:

1.	Hot air oven	chamber size minimum 24"x 24" x 24"
2.	Rotary shaker	
3.	Vacuum filtration device	
4.	Vacuum pump	
5.	pH meter	
6.	Electronic Weighing balance	
7.	Conductivity meter	
8.	Flame Photometer	
9.	Spectrophotometer	360-960nm
10.	Muffel furnace	
11.	Hot plate cum stirrer	
12.	Atomic Absorption Spectrophotometer (AAS)	
13.	Cold vapour Mercury analyzer or vapour generator Assembly for AAS	
14.	Kjedahl digestion unit	
15.	Kjeldahl distillation assembly	
16.	Fume hood with exhaust facility	
17.	Soxhlet apparatus for refluxing	