

**MINISTRY OF AGRICULTURE**  
**(Department of Agriculture and Co-operation)**  
**ORDER**

New Delhi, the 22nd June, 2012

**S.O. 1420(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 further to amend the Fertilizer (Control) Order, 1985, namely:-

1. (1) This Order may be called the Fertilizer Control (Amendment) Order, 2012.  
 (2) It shall come into force on the date of its publication in the Official Gazette.
  
  2. In Fertilizer (Control) Order, 1985,-
    - (A) in Schedule I, in Part A, under the heading "SPECIFICATIONS OF FERTILISERS",-
      - (i) in sub-heading "1 (d) N.P. COMPLEX FERTILISERS",-
        - (a) serial number "12. Nitrophosphate (23- 23- 0)" and the entries relating thereto, shall be omitted;
        - (b) after serial number 17 and the entries relating thereto, the following serial number and entries shall be inserted, namely:-
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- "18. Nitrophosphate (24:24:0)
 

(i) Moisture per cent. by weight, maximum	1.5
(ii) Total nitrogen per cent. by weight, minimum	24.0
(iii) Nitrogen in ammonical form per cent. by weight, minimum	13.5
(iv) Nitrogen in nitrate form, per cent. by weight, maximum	10.5
(v) Neutral ammonium citrate soluble phosphate (as P <sub>2</sub> O <sub>5</sub> ) per cent. by weight, minimum	24.0
(vi) Water soluble phosphate (as P <sub>2</sub> O <sub>5</sub> ) per cent. by weight, minimum	20.5
(vii) Particle size: Not less than 90 per cent. of the material shall pass through 4mm IS sieve and be retained on 1mm IS sieve. Not more than 5 per cent shall be below 1mm IS sieve."	
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- (ii) in sub-heading "1 (f) MICRONUTRIENTS", in serial number "8 Chelated Zinc as Zn-EDTA", for the words, "Appearance- free flowing crystalline/powder" the words "Appearance- free flowing crystalline or powder or tablet" shall be substituted
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- (iii) in sub-heading "1 (g) FORTIFIED FERTILISERS",-
  - (a) for serial number 3 and the entries relating thereto, the following serial number and entries shall be substituted, namely:-

"3. Zincated Phosphate (Suspension) – for seed treatment

(i)	Total phosphate (as P <sub>2</sub> O <sub>5</sub> ) per cent. by weight, minimum.	13.9
(ii)	Total zinc (as Zn) per cent. by weight, minimum.	17.6
(iii)	Neutral ammonium citrate soluble phosphate (as P <sub>2</sub> O <sub>5</sub> ) per cent. by weight, minimum	2.8
(iv)	Lead ( as Pb) per cent. by weight, maximum	0.003
(v)	pH	8+/-1";

(b) after serial number 10 and the entries relating thereto, the following serial number and entries shall be inserted, namely:-

"11. DAP fortified with Zinc (18:46:0:0.5)

(i)	Moisture per cent. by weight, maximum.	2.5
(ii)	Total nitrogen per cent. by weight, minimum	18.0
(iii)	Ammonical nitrogen per cent. by weight, minimum	15.5
(iv)	Urea nitrogen percent. by weight, maximum	2.5
(v)	Neutral ammonium citrate soluble phosphate (as P <sub>2</sub> O <sub>5</sub> ) per cent. by weight, minimum	46.0
(vi)	Water soluble phosphate (as P <sub>2</sub> O <sub>5</sub> ) per cent. by weight, minimum	41.0
(vii)	Zinc (as Zn ) per cent. by weight, minimum	0.5
(viii)	Particle size: Not less than 90 per cent of the material shall pass through 4mm IS sieve and be retained on 1mm IS sieve. Not more than 5 per cent shall be below 1mm IS sieve."	

(iv) in sub-heading "1 (h) 100% WATER SOLUBLE COMPLEX FERTILISER", after serial number 16 and the entries relating thereto, the following serial number and entries shall be inserted, namely:-

"17 NPKZn (7.6 : 23.5 :7.6 :3.5)

(i)	Moisture per cent. by weight, maximum	0.5
(ii)	Total nitrogen per cent. by weight, minimum	7.6
(iii)	Nitrate nitrogen per cent. by weight, maximum	2.8
(iv)	Ammonical nitrogen per cent. by weight, minimum	5.0
(v)	Water soluble phosphate (as P <sub>2</sub> O <sub>5</sub> ) per cent. by weight minimum	23.5
(vi)	Water Soluble Potash (K <sub>2</sub> O ) per cent. by weight, minimum	7.6
(vii)	Water Soluble Zinc (as Zn EDTA) per cent. by weight, minimum	3.5
(viii)	Sodium (as NaCl) per cent. by weight, on dry basis maximum	0.15
(ix)	Matter insoluble in water per cent. by weight, maximum";	0.5

(B) in Schedule III, -

(i) in Part A, under the heading "SPECIFICATIONS OF BIOFERTILIZERS" after serial number 5 and the entries relating thereto, the following serial number and entries shall be inserted, namely:-

**"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 $5 \times 10^7$ cells/g of powder, granules or carrier material on dry weight basis or $1 \times 10^8$ 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	Minimum 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 $5 \times 10^7$ cells/g of powder, granules or carrier material on dry weight basis or $1 \times 10^8$ 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 content percent. by weight, maximum in case of carrier based	30 – 40
7.	Efficiency character	Minimum 10 mm solubilization zone in prescribed media having at least 3mm thickness."

(ii) in PART D, under the heading "METHODS OF ANALYSIS OF BIOFERTILISERS", after serial number 1E and entries relating thereto, the following entries shall be inserted, namely:-

“IF. Method of analysis for Potash Solubilizing 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, calibrated to read upto 250°C and with vent 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 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
Potassium mineral (mica powder)	2.0
Calcium phosphate	2.0
Distilled water	1000 ml

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## 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°C for not less than 2 hours;

2.2.2 Sterilize the media by autoclaving at 120°C for 20 min. To permit passage of steam into and from closed containers when auto claved, keep stoppers slightly loosened 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-sterilisation 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°C, and store until used, in a water bath or incubator at 43 to 45°C. Introduce 12 to 15 ml of liquefied medium or appropriate quantity depending on size of the petri dish at 42 to 44°C into each plate. Gently lift the cover of the dish just enough to pour in the medium. Sterilise 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 plates. 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 demineralized 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.

## 3. Incubation of Plates:

4.1 Label the plates and incubate at  $28 \pm 2^\circ\text{C}$  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. *Frutaria 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. Counting

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

#### 5. Method for estimation of K solubilization zones

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

6.2 Make a tenfold dilution series up to  $10^7$ .

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

6.4 Spread it uniformly, Invert the plates and incubate for up to 2 weeks at  $28 \pm 2^\circ\text{C}$ .

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

### 1G. Method of analysis for Zinc Solubilizing Biofertilizers

#### 2. 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, calibrated to read upto  $250^\circ\text{C}$  and with vent 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 Medium

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

Medium for analysis of Total Viable Count, Contamination and zone of solubilisation for Zn solubilizing 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

## 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°C for not less than 2 hours;

2.2.2 Sterilize the media by autoclaving at 120°C for 20 min. To permit passage of steam into and from closed containers when auto claved, keep stoppers slightly loosened 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 hours. 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°C, and store until used, in a water bath or incubator at 43 to 45°C. Introduce 12 to 15 ml of liquefied medium or appropriate quantity depending on size of the petri dish at 42 to 44°C into each plate. Gently lift the cover of the dish just enough to pour in the medium. Sterilise 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 plates. 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 de-mineralized 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 used droplet method. Invert the plates and promptly place them in the incubator.

#### 4. Incubation of Plates:

4.1 Label the plates and incubate at  $28 \pm 2^\circ\text{C}$  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.

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. Zinc solubilising 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 for estimation of Zinc solubilisation zones

6.1 Take 10 g of ZSB in 90 ml sterile distilled water

6.2 Make a tenfold dilution series up to  $10^7$ .

6.3 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  $28 \pm 2^\circ\text{C}$ .

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

(D) In Schedule IV, in PART A, under the heading "SPECIFICATIONS OF ORGANIC FERTILISERS", after serial number 2 and the entries relating thereto, the following serial number and entries shall be inserted, namely: -

#### "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 ( $\text{g}/\text{cm}^3$ )	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 $\text{P}_2\text{O}_5$ ) per cent. by weight, minimum	10.42
(vii)	Total potash (as $\text{K}_2\text{O}$ ) per cent. by weight, minimum	-

(viii)	C: N ratio	18.73:1
(ix)	pH (1:5 solution) maximum	6.72
(x)	Conductivity (as $\text{dSm}^{-1}$ ) not more than	8.27
(xi)	Heavy metal content (as mg/kg), maximum	
	Arsenic (as $\text{As}_2\text{O}_3$ )	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

[F. No. 2-1/2012-Fert.Law]

NARENDRA BHOOSHAN, Jt. Secy.

**Note :—**The principal order was published in the Gazette of India, Extraordinary, Part II, section 3, sub-section (i) vide number G.S.R. No. 758(E) dated 25<sup>th</sup> September, 1985 and was subsequently amended vide S.O. No. 2203 dated 22<sup>nd</sup> September, 2011

