



BSI Standards Publication

Soil improvers and growing media — Determination of plant response

Part 1: Pot growth test with Chinese cabbage

National foreword

This British Standard is the UK implementation of EN 16086-1:2011.

BSI, as a member of CEN, is obliged to publish EN 16086-1:2011 as a British Standard. However, attention is drawn to the fact the UK committee voted against its approval as a European Standard.

The UK committee voted against the publication of this standard because the plant species specified for the test are not sensitive to some of the pesticides of greatest concern currently in composted materials, and because it considered that the reproducibility obtained in inter-laboratory evaluation was not an adequate basis on which to establish a reference method. This could cause problems in the event of dispute or litigation.

The standard deviations of reproducibility in the inter-laboratory validation trials of this method were poor and there is a low probability of getting the same result from two laboratories analysing the same sample. In the worst cases the standard deviation of reproducibility was about half the mean analysis (after rejection of outliers); for example the mean weight of plants per pot in compost 1 was 12.36 g, S_R 6.95.

The UK committee advises that this standard will not detect inhibition by residues of some of the herbicides of greatest concern, and that in addition the results are imprecise.

The UK participation in its preparation was entrusted to Technical Committee AW/20, Top soil and other growing media.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Compliance with a British Standard cannot confer immunity from legal obligations.

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Détermination de la réponse des plantes - Partie 1: Essai
de croissance en pot avec du chou de Chine

Bodenverbesserungsmittel und Kultursubstrate -
Bestimmung der Pflanzenverträglichkeit - Teil 1:
Wachstumstest mit Chinakohl im Topf

This European Standard was approved by CEN on 17 September 2011.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN 16086-1:2011) has been prepared by Technical Committee CEN/TC 223 "Soil improvers and growing media", the secretariat of which is held by ASI.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2012, and conflicting national standards shall be withdrawn at the latest by May 2012.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard describes a method for the routine determination of the effect of soil improvers and growing media or constituents thereof on the growth of Chinese cabbage (and in certain cases spring barley).

This test may not be suitable for all growing media since the growing media characteristics (e.g. nutrient content) will vary according to target use and the product is not tested in accordance with the specified use and pack recommendations.

This test is not appropriate for the detection of nitrogen immobilization.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 13037, *Soil improvers and growing media – Determination of pH*

EN 13038, *Soil improvers and growing media – Determination of electrical conductivity*

EN 13040, *Soil improvers and growing media – Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density*

EN ISO 3696, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

plant response

variation in plant germination and/or growth when sown and grown in a growing medium, soil improver or constituent thereof or in an extract obtained from these materials

Factors causing negative plant growth cannot be identified nor quantified by applying this method.

3.2

prepared sample

portion of the laboratory sample, undiluted or diluted with sphagnum peat at given ratios, fertilized and limed as required

4 Principle of the plant growth response test

4.1 General

Depending on the material to be tested, one of the two methods described in this standard shall be used.

4.2 Pot experiment with direct use of the prepared sample

Sowing a defined quantity of Chinese cabbage into pots containing the prepared sample, cultivating under controlled conditions for a defined period of time and evaluating the plant response by determining the germination rate, fresh weight, abnormalities and overall plant growth.

If the presence of graminaceous herbicides is suspected, Spring barley shall be used in addition to Chinese cabbage. For testing of other specific effects, the use of additional plant species (for example lettuce) can be considered.

4.3 Pot experiment using an extract of the original sample

Mixing the original sample with nutrient solution as an extractant, soaking for 4 h at ambient temperature and collecting the freely available nutrient solution. Filling pots with perlite saturated with the extract and continuing as described under 4.2, irrigating during the test period with a fixed quantity of the extract and afterwards water.

If the presence of graminaceous herbicides is suspected, Spring barley shall be used in addition to Chinese cabbage. For testing of other specific effects, the use of additional plant species (for example lettuce) can be considered.

5 Choice of methodology

For most materials, the pot growth test can be carried out as described in Clause 6. However, for coarse materials such as bark, expanded clay, lava, mineral wool, perlite, polyurethane and pumice with an inherently low water holding capacity which are used as growing media without amendment, this procedure is unsuitable and the extract method described in Clause 7 shall be adopted.

6 Pot experiment with direct use of the prepared sample

6.1 Materials

6.1.1 Water of class 3

According to EN ISO 3696.

6.1.2 Sphagnum peat

Sphagnum peat with a degree of humification of H3 – H5 according to von Post scale, having a pH measured according to EN 13037 of between 3,0 and 4,5, an EC measured according to EN 13038 of between 1 mS m⁻¹ and 5 mS m⁻¹, a particle size of < 10 mm and to which neither lime nor fertilizer has been added.

6.1.3 Fertilized and limed sphagnum peat

Sphagnum peat (see 6.1.2), having a pH measured according to EN 13037 adjusted using ground limestone (see 6.1.4) to a range between 5,5 and 6,5, fertilized with a water soluble “complete” fertilizer with essential

micronutrients, added at a rate to supply $(225 \pm 25) \text{ mg N} \cdot \text{l}^{-1}$, (for example $1,5 \text{ g} \cdot \text{l}^{-1}$ water soluble complete fertilizer N : P₂O₅ : K₂O – 15 : 10 : 20), (see B.1).

6.1.4 Ground limestone

Finely ground limestone, containing at least 5 % MgCO₃, having a particle size less than 1 mm and a moisture content of less than 1 % m/m.

6.1.5 Seeds of Chinese cabbage (*Brassica napa*, ssp. *pekinensis*)

Specified germination capacity $\geq 95 \%$.

6.1.6 Seeds of Spring barley (*Hordeum vulgare*)

Specified germination capacity $\geq 95 \%$.

6.2 Apparatus

6.2.1 Sieve with 20 mm mesh size

6.2.2 Sieve with 5 mm mesh size

6.2.3 Circular plant pot

Upper diameter $(12 \pm 0,5) \text{ cm}$, height between 8,5 cm and 9,0 cm, volume between 650 ml and 700 ml, perforated bottom to provide drainage (for example plastic pot used in horticulture).

6.2.4 Saucer

Saucer capable of catching all surplus water from the plant container after overhead watering.

6.2.5 Thin fleece or plastic sheet for covering the containers

6.2.6 Testing facility

Testing facility capable of maintaining and monitoring the temperature and light intensity specified in 6.4 such as a greenhouse or plant growth room.

6.2.7 Irrigation device for watering the pots

For example watering can, greenhouse watering hose

6.2.8 Analytical balance

Accuracy 0,01 g, capacity 500 g.

6.3 Preparation of the sample

6.3.1 General preparation

Pass the sample through a 20 mm sieve (see 6.2.1). Any foreign material such as plastic, metal or glass retained on the sieve shall be removed and noted. Other material that is retained on the sieve and which is an intrinsic part of the sample shall be physically reduced to parts of similar size as few times as are necessary to permit the entire sample to pass through the sieve. Fibrous materials i.e. coir fibres and straw shall be cut to a length $\leq 20 \text{ mm}$ by using scissors. Thoroughly mix the laboratory sample with the broken particles that had been retained on the sieve taking care to minimise physical damage to the sample as a whole. Transportation

and possible storage of the samples shall be done in accordance with EN 13040, using food grade polyethylene bags.

6.3.2 Mixing procedure

Before mixing, the laboratory compacted bulk density shall be measured (according to EN 13040).

To produce the prepared sample, the material prepared as described in 6.3.1 is thoroughly mixed with sphagnum peat (see 6.1.2) on a volume/volume – basis as given in Table 1. The pH according to EN 13037 is ideally within the range between 5,5 and 6,5. If it is below, the pH shall be adjusted by adding limestone (see 6.1.4). If it is above, the pH shall be noted.

Additionally, a water soluble complete fertilizer with essential micronutrients, added at a rate to supply $(225 \pm 25) \text{ mg N} \cdot \text{l}^{-1}$ (for example $1,5 \text{ g} \cdot \text{l}^{-1}$ water soluble complete fertilizer N : P₂O₅ : K₂O – 15 : 10 : 20) (see B.1) is added to one litre of prepared sample (according to EN 13040).

The dilution ratios in Table 1 are based on horticultural practice in the usage of growing media, growing media constituents, soil improvers and soil improver constituents. In general, it is sufficient to carry out the test using the highest proportion of test material. The second dilution ratio may be helpful for further judging plant response.

If required (for example to fulfil certain quality certification requirements or legislation), materials can be tested with other dilution ratios than mentioned in Table 1 or without dilution. It might also be necessary to apply further nutritional adjustments, cases are described in B.2. Any adjustments shall be reported.

Table 1 — Dilution ratios for test materials (growing media and constituents thereof; soil improvers and constituents thereof), using sphagnum peat (see 6.1.2) as the dilution material for pot growth tests with Chinese cabbage

Test material	Ratio (V/V) of test material to sphagnum peat to obtain the prepared sample ^a	
Growing media		
All kinds (except pre-shaped growing media and roof-garden media)	100 : 0	
Organic materials		
	obligatory	optional
Bark (uncomposted)	50 : 50	25 : 75
Brown coal (lignite)	50 : 50	25 : 75
Cocoa hulls	50 : 50	25 : 75
Coir pith	100 : 0	50 : 50
Coir fibres	50 : 50	25 : 75
Coir chips	50 : 50	25 : 75
Composts made of material such as biodegradable waste, bark, wood, straw, manure	50 : 50	25 : 75
Forest litter	50 : 50	25 : 75
Manure	25 : 75	10 : 90
Peat (any type)	100 : 0	50 : 50
Rice hulls	50 : 50	25 : 75
Solid digestate	20 : 80	10 : 90
Spent mushroom casing soil	25 : 75	10 : 90
Straw	50 : 50	25 : 75
Wood fibres	50 : 50	25 : 75
Wood chips	50 : 50	25 : 75
Wood shavings	50 : 50	25 : 75
Mineral materials		
Clay	25 : 75	10 : 90
Expanded clay (also broken)	50 : 50	25 : 75
Lava	50 : 50	25 : 75
Mineral wool flakes	50 : 50	25 : 75
Perlite (expanded)	50 : 50	25 : 75
Pumice	50 : 50	25 : 75
Sand	50 : 50	25 : 75
Vermiculite	50 : 50	25 : 75
Synthetic-organic materials (plastics)		
Expanded polystyrene flakes (styrofoam), urea-formaldehyde foam resins, etc.	50 : 50	25 : 75
NOTE Non-listed materials may also be tested; the dilution ratio(s) for non-listed materials may be chosen by analogy with similar product groups listed in Table 1.		
^a To obtain the required quantity of test material and sphagnum peat for example at a ratio of 25 % (V/V) : 75 % (V/V), take – for example – one litre of test material and three litres of sphagnum peat. The laboratory compacted bulk density of the materials according to EN 13040 shall be the basis.		

6.4 Test procedure

To obtain a sufficient quantity of fine material to cover the seeds (see below), a portion of the prepared sample is sieved < 5 mm. The remaining part of the prepared sample shall be moistened to the approximate optimum moisture content according to the fist test (see B.3).

Fill three pots (see 6.2.3) with the prepared sample in the following way:

Fill each pot with the prepared sample to the rim, drop it three times from a height of (3 ± 2) cm and top it up again to the rim. Then compress the prepared sample with a round plate or saucer until the surface is 5 mm to 10 mm below the rim of the pot. If an even surface is not obtained due to coarse particles, fill spaces with mixed sample material passing through a 5 mm sieve (see 6.2.2).

Place each pot on a saucer (see 6.2.4).

NOTE 1 A higher number of replicates may be used. The number of replicates should be taken into account for the calculation of the results

Spread 20 seeds of Chinese cabbage (see 6.1.5) evenly on the surface of each of three pots of the prepared sample and cover them with a thin layer (approximately 2 mm to 5 mm) of prepared sample passing through a 5 mm sieve (see 6.2.2). Then gently compact the surface again by using a suitable flat, round plate or saucer and moisten with water using a fine sprayer to ensure contact between the seed and the test material.

NOTE 2 To cover the Spring barley seeds (see 6.1.6), usually more material (approximately 5 mm to 10 mm) than for cabbage is needed.

The pots shall be arranged in the test area in a randomized design to minimise positional effects and bias associated with variations in temperature, light or humidity which affect plant growth.

Then, the pots in the testing facility (see 6.2.6) at a temperature maintained between 18 °C and 30 °C are loosely covered with horticultural fleece or plastic sheet (see 6.2.5) until at least 50 % of the seeds have germinated. After uncovering the pots the light intensity shall be maintained at 10 W m^{-2} for 12 h to 16 h per day and the sample kept moist by overhead irrigation with water (see 6.1.1). The watering intervals are dependent on plant growth and environmental conditions in accordance with good horticulture practice. Alternatively, a watering process according to ISO 22030 is possible.

NOTE 3 Steps should be taken to avoid over-watering and water logging of the test sample.

NOTE 4 To improve the watering process, the pots can be weighed during the test to provide an indication of the moisture content of the test samples and guidance about the frequency and quantity of water that needs to be applied to keep them moist.

6.5 Control sample

As a control, the procedure as described above is carried out using fertilized and limed sphagnum peat (see 6.1.3).

6.6 Validity of the test

If the average germination in the control sample (see 6.5) is below 85 % after five days, the test is not valid.

6.7 Evaluation parameters

The following parameters are monitored:

6.7.1 Germination rate (%)

After five days, the number of germinated seeds is recorded. A seed is germinated as soon as approximately 50 % of the cotyledon surface is visible.

- **Calculation of the germination rate per pot (*GR*):** Calculate the germination as a percentage of the total sown seeds per pot according to Equation (1).

$$GR = \frac{NGS}{20} \cdot 100 \quad (1)$$

where

GR is the germination rate;

NGS is the number of germinated seeds.

Calculation of the average germination rate and the coefficient of variance: Calculate the average germination rate (*AGR*) from the results of the different pots and the coefficient of variation according to Equations (2) and (3).

$$\text{Average Germination rate (AGR, \%)} = \frac{\sum_{n=1}^n GR(\text{pot}_n)}{n} \quad (2)$$

where

AGR is the average germination rate.

$$CVG = \frac{\sqrt{\frac{\sum_{n=1}^n (GR_n - AGR)^2}{n-1}}}{AGR} \times 100 \quad (3)$$

where

CVG is the coefficient of variance for the germination rate.

Calculate the germination rate and the coefficient of variance for the control sample in the same way.

Calculation of the germination inhibition (*GeI*): The inhibition of germination is expressed as a percentage of the average germination in the control according to Equation (4)

$$GeI (\%) = \frac{AGR_{\text{control}} - AGR_{\text{sample}}}{AGR_{\text{control}}} \times 100 \quad (4)$$

where

GeI is the germination inhibition.

6.7.2 Fresh weight

Determine the total weight and number of plants in each pot as soon as the fifth true leaf is clearly visible on at least 50 % of the plants of the control sample.

The growing medium shall be watered thoroughly to ensure that the plants are turgid before harvesting, taking care not to wet the leaves as these must be dry when harvested.

NOTE If using spring barley, the fresh weight is monitored as soon as the second true leaf of 50 % of the plants of the control sample are bigger than the first leaf.

For harvest, the plants are cut at substrate level.

Calculation of the average weight of plants per pot: Calculate the average weight of plants per pot (W) in grams according to Equation (5)

$$W = \frac{\sum WP (g)}{3} \quad (5)$$

where

W is the average weight of plants, per pot;

WP is the weight of plants, per pot.

Calculation of the average plant weight per pot: Calculate the average plant weight in grams according to Equation (6)

$$PW = \frac{WHP (g)}{NHP} \quad (6)$$

where

PW is the average plant weight, per pot;

WHP is the weight of harvested plants, per pot;

NHP is the number of harvested plants, per pot.

Calculation of the average plant weight and the coefficient of variance: Calculate the average plant weight (APW) from the results of the different pots and the coefficient of variation according to Equations (7) and (8).

$$APW = \frac{PW(\text{pot 1}) + PW(\text{pot 2}) + PW(\text{pot 3})}{3} \quad (7)$$

where

APW is the average plant weight.

$$CVP = \frac{\sqrt{\frac{\sum (PW - APW)^2}{2}}}{APW} \times 100 \quad (8)$$

where

CVP is the coefficient of variance for the plant weight.

Calculate the average and the coefficient of variance for the control sample in the same way.

Calculate the difference between the results for the control sample and the test sample according to the following equations:

$$DW = W_{\text{control}} - W_{\text{sample}} \quad (9)$$

where

DW is the difference of average weight of plants, per pot;

W_{control} is the average weight of plants per pot of the control sample;

W_{sample} is the average weight of plants per pot of the test sample.

$$DP = APW_{\text{control}} - APW_{\text{sample}} \quad (10)$$

where

DP is the difference of average plant weight;

APW_{control} is the average plant weight of the control sample;

APW_{sample} is the average plant weight of the test sample.

6.7.3 Growth inhibition

6.7.3.1 Calculation of the growth inhibition (GrI): Calculate the growth inhibition from to the difference of the plant weight of the control and the sample as a percentage according to Equation (11).

$$GrI (\%) = \frac{APW_{\text{control}} - APW_{\text{sample}}}{APW_{\text{control}}} \times 100 \quad (11)$$

where

GrI is the growth inhibition.

6.7.3.2 Calculation of the growth inhibition per pot ($GrIP$): Calculate the growth inhibition from the difference of the plant weight per pot of the control and the sample as a percentage according to Equation (12).

$$GrIP (\%) = \frac{WP_{\text{control}} - WP_{\text{sample}}}{WP_{\text{control}}} \times 100 \quad (12)$$

where

$GrIP$ is the growth inhibition, per pot.

6.7.4 Abnormalities in comparison to the control

Symptoms to be described as precise as possible with reference to for example:

- leaf colour and morphology,
- shoot morphology,
- roots colour and morphology,
- roots death.

7 Pot experiment using an extract of the original sample

7.1 Materials

7.1.1 Water of class 3

According to EN ISO 3696.

7.1.2 Perlite

Particle size < 2,5 mm, maximum 20 % W/W < 0,5 mm.

NOTE A small quantity of water should be added to avoid disintegration of the particles.

7.1.3 Seeds of Chinese cabbage (*Brassica napa*, ssp. *pekinensis*)

Specified germination capacity \geq 95 %.

7.1.4 Seeds of Spring barley (*Hordeum vulgare*)

Specified germination capacity \geq 95 %.

7.1.5 Balanced nutrient solution (see B.1)

Adjust the pH of the solution (see B.1) to reach 5,5 to 6,5, using 1M nitric acid (see 7.1.6).

7.1.6 Nitric acid (HNO₃), 1M

7.2 Apparatus

7.2.1 Sieve with 20 mm mesh size

7.2.2 Circular plant pot

Upper diameter (12 \pm 0,5) cm, height between 8,5 cm and 9,0 cm, volume between 650 ml and 700 ml, perforated bottom to provide drainage.

NOTE A plastic plant pot as used in horticulture is recommended.

7.2.3 Saucer

Saucer capable of catching all surplus water from the plant container after overhead watering.

7.2.4 Bucket

Plastic bucket, for example approximately 30 cm diameter on top, height approximately 20 cm, volume between 10 l and 12 l.

NOTE A mesh or fleece may be used for covering the bucket in case of floating materials.

7.2.5 Thin fleece or plastic sheet for covering the containers

7.2.6 Testing facility

Testing facility capable of maintaining and monitoring the temperature and light intensity specified in 6.4 such as a greenhouse or plant growth room.

7.2.7 Irrigation device for watering the pots

For example watering can, greenhouse watering hose.

7.2.8 Analytical balance

Accuracy 0,01 g, capacity 500 g.

7.3 Preparation of the sample

7.3.1 General preparation

Pass the sample through a 20 mm sieve (see 7.2.1). Any foreign material such as plastic, metal or glass retained on the sieve shall be removed and noted. Other material that is retained on the sieve and which is an intrinsic part of the sample shall be physically reduced to parts of similar size as few times as are necessary to permit the entire sample to pass through the sieve. Fibrous materials i.e. coir fibres and straw shall be cut to a length of ≤ 20 mm by using scissors. Thoroughly mix the laboratory sample with the broken particles that had been retained on the sieve taking care to minimise physical damage to the sample as a whole. Transportation and possible storage of the samples shall be done in accordance with EN 13040, using food grade polyethylene.

7.3.2 Extraction procedure

Take a bucket (see 7.2.4) of appropriate volume (appropriate for the type of growth response test to follow).

Fill the bucket evenly to the rim with the sieved material and compact by dropping the bucket 3 times from (3 ± 1) cm height. Add nutrient solution (see 7.1.5) slowly until the material is just covered and homogenize. If the material floats, cover the top with a weight (for example bucket of the same size filled with some water) to ensure that the material is submerged.

Leave for 4 h at ambient temperature.

Collect the free extract by decanting.

7.4 Test procedure

Fill three pots (see 7.2.2) with perlite (see 7.1.2) in the following way:

Fill each pot with perlite (see 7.1.2) to the rim, drop it three times from a height of (3 ± 2) cm and top it up again to the rim. Then compress the prepared sample with a round plate or saucer until the surface is 5 mm to 10 mm below the rim of the pot. If a further compression after the tamping is not possible, remove some of the perlite until the perlite surface is 5 mm to 10 mm below the pot rim. Slowly irrigate the perlite with 400 ml extract until it is saturated and the solution drains out from the bottom of the pot.

Place each pot on a saucer (see 7.2.3).

As perlite retains less water than many other substrates, irrigation has to be performed more frequently and in smaller doses than in Clause 6 – direct use of the material.

BS EN 16086-1:2011
EN 16086-1:2011 (E)

NOTE 1 A higher number of replicates can be used. The number of replicates should be taken into account for the calculation of the results.

Spread evenly 20 seeds of Chinese cabbage (see 7.1.3) on the surface of each of three pots of the perlite and cover them with a thin layer (approximately 2 mm) of perlite. The surface is again gently compacted by using the plate or saucer and moistened with extract.

NOTE 2 To cover the barley seeds, usually more material (approximately 5 mm) than for cabbage is needed.

The pots shall be arranged in the test area in a randomized design. Then, the pots are loosely covered with horticultural fleece or plastic sheet (see 7.2.5) until germination and kept in the testing facility (see 7.2.6) at a temperature suitable for plant germination (range between 18 °C and 30 °C) at a minimum light intensity of 10 W m⁻² for 12 h to 16 h per day. After germination, the sample has to be kept moist by overhead irrigation with water (see 7.1.1). The watering intervals are dependent on plant growth and environmental conditions in accordance with good horticulture practice. Start the watering with the remaining extract in the saucer, continuing with water after using up the extract.

NOTE 3 Steps should be taken to avoid excess water.

7.5 Control sample

As a control, the procedure as described in 7.4 is carried out using pure nutrient solution (see 7.1.5) to irrigate perlite.

7.6 Validity of the test

If the average germination in the control sample (see 7.5) is below 85 % after five days, the test is not valid.

7.7 Evaluation parameters

The parameters to be monitored and respective calculations are listed under 6.7.

8 Precision

No data available yet.

9 Test report

The test report shall include the following information:

- a reference to this European Standard,
- a complete identification of the sample,
- indication of the method used,
 - pH (if above 6,5),
 - dilution ratios,
 - specify pot or extraction method,
 - fertilizers and nutrient solutions used,
 - any adjustments with regard to mixing ratios or fertilization,

- average germination rate of both control and test sample(s),
- average inhibition of germination and coefficient of variance,
- average fresh weight of plants per pot of the test sample and the control,
- average number of harvested plants per pot of the test sample and the control,
- average fresh weight per plant of the test sample and the control,
- average inhibition of growth per pot,
- abnormalities in comparison to the control.

Annex A (informative)

Validation

Samples:

PBGM 1 (I): peat based growing medium no. 1, direct use

PBGM 1 (II): peat based growing medium no. 1, extract method

PBGM 2: peat based growing medium no. 2, direct use

Bark (I): uncomposted bark, direct use, dilution rate 25 : 75

Bark (I): uncomposted optional: bark optional, direct use, dilution rate 50 : 50

Bark (II): uncomposted bark, extract method

Compost 1: compost no. 1, direct use

Compost 2: compost no. 2, direct use

In Table A.1 to Table A.7, the statistical results of the interlaboratory test are given.

Table A.1 — Summary of the results of the pot growth test – *AGR* (germination rate)

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I) optional	bark (I)	bark (II)	compost 1	compost 2
	%							
Number of laboratories retained after eliminating outliers	11	10	11	12	8	10	12	11
Number of outliers (laboratories)	0	0	1	1	1	0	0	1
Mean value	95,46	89,50	95,46	92,36	93,54	89,17	72,36	87,73
Repeatability standard deviation, s_r	5,44	9,87	7,22	7,12	6,80	10,25	6,40	7,45
Repeatability relative standard deviation	0,06	0,11	0,08	0,08	0,07	0,12	0,09	0,09
Repeatability limit, $r = 2,8 s_r$	15,22	27,65	20,21	19,93	19,05	28,69	17,92	20,87
Reproducibility standard deviation, s_R	0,87	6,64	13,83	16,75	6,38	4,94	33,34	26,24
Reproducibility relative standard deviation	0,01	0,07	0,15	0,18	0,07	0,06	0,46	0,30
Reproducibility limit, $r = 2,8 s_R$	2,44	18,59	38,71	46,90	17,86	13,84	93,36	73,48

Table A.2 — Summary of the results of the pot growth test – *GeI* (germination inhibition)

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I) optional	bark (I)	bark (II)	compost 1	compost 2
	%							
Number of laboratories retained after eliminating outliers	10	9	11	13	8	10	12	11
Number of outliers (laboratories)	1	1	1	0	1	0	0	1
Mean value	-5,75	2,93	0,89	1,30	3,44	0,12	20,50	1,11
Repeatability standard deviation, s_r	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability relative standard deviation	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability limit, $r = 2,8 s_r$	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Reproducibility standard deviation, s_R	16,16	6,72	14,40	5,43	4,47	5,81	18,96	18,42
Reproducibility relative standard deviation	-2,81	2,29	16,24	4,20	1,30	49,04	0,93	16,59
Reproducibility limit, $r = 2,8 s_R$	45,25	18,82	40,33	15,20	12,53	16,26	53,09	51,57

Table A.3 — Summary of the results of the pot growth test – W (average weight per pot)

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I) optional	bark (I)	bark (II)	compost 1	compost 2
	g							
Number of laboratories retained after eliminating outliers	11	10	12	13	9	10	12	12
Number of outliers (laboratories)	0	1	0	0	0	0	0	0
Mean value	22,53	17,30	18,79	17,57	19,43	11,83	12,36	14,70
Repeatability standard deviation, s_r	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability relative standard deviation	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability limit, $r = 2,8 s_r$	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Reproducibility standard deviation, s_R	8,47	5,25	7,92	6,87	7,05	3,76	6,95	7,13
Reproducibility relative standard deviation	0,38	0,30	0,42	0,39	0,36	0,32	0,56	0,49
Reproducibility limit, $r = 2,8 s_R$	23,71	14,70	22,18	19,24	19,74	10,52	19,45	19,95

Table A.4 — Summary of the results of the pot growth test – DW

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I) optional	bark (I)	bark (II)	compost 1	compost 2
	g							
Number of laboratories retained after eliminating outliers	11	10	12	13	8	9	12	12
Number of outliers (laboratories)	0	0	0	0	1	1	0	0
Mean value	1,89	-3,41	5,75	7,56	4,33	1,73	12,19	9,85
Repeatability standard deviation, s_r	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability relative standard deviation	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability limit, $r = 2,8 s_r$	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Reproducibility standard deviation, s_R	5,18	4,53	4,66	5,92	4,95	5,44	5,64	4,83
Reproducibility relative standard deviation	2,74	-1,33	0,81	0,78	1,14	3,14	0,46	0,49
Reproducibility limit, $r = 2,8 s_R$	14,50	12,68	13,06	16,56	13,86	15,23	15,80	13,53

Table A.5 — Summary of the results of the pot growth test – DP

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I) optional	bark (I)	bark (II)	compost 1	compost 2
	g							
Number of laboratories retained after eliminating outliers	11	9	12	13	7	9	12	12
Number of outliers (laboratories)	0	1	0	0	1	1	0	0
Mean value	0,17	-0,33	0,32	0,41	0,22	0,07	0,62	0,48
Repeatability standard deviation, s_r	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability relative standard deviation	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability limit, $r = 2,8 s_r$	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Reproducibility standard deviation, s_R	0,25	0,34	0,47	0,59	0,46	0,71	0,53	0,43
Reproducibility relative standard deviation	1,47	-1,03	1,45	1,45	2,10	10,28	0,86	0,91
Reproducibility limit, $r = 2,8 s_R$	0,70	0,94	1,32	1,64	1,29	1,97	1,49	1,21

Table A.6 — Summary of the results of the pot growth test – *GrI* (growth inhibition)

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I) optional	bark (I)	bark (II)	compost 1	compost 2
	%							
Number of laboratories retained after eliminating outliers	11	10	12	13	9	10	12	12
Number of outliers (laboratories)	0	0	0	0	0	0	0	0
Mean value	7,81	-53,86	14,73	18,93	13,70	9,14	39,03	25,37
Repeatability standard deviation, s_r	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability relative standard deviation	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability limit, $r = 2,8 s_r$	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Reproducibility standard deviation, s_R	16,55	50,00	22,98	22,27	17,62	21,55	45,69	18,81
Reproducibility relative standard deviation	2,12	-0,93	1,56	1,18	1,29	2,36	1,17	0,74
Reproducibility limit, $r = 2,8 s_R$	46,33	140,01	64,35	62,36	49,34	60,34	127,93	52,67

Table A.7 — Summary of the results of the pot growth test – *GrIP* (growth inhibition per pot)

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I) optional	bark (I)	bark (II)	compost 1	compost 2
	%							
Number of laboratories retained after eliminating outliers	11	10	12	13	9	10	12	12
Number of outliers (laboratories)	0	0	0	0	0	0	0	0
Mean value								
Repeatability standard deviation, s_r								
Repeatability relative standard deviation								
Repeatability limit, $r = 2,8 s_r$								
Reproducibility standard deviation, s_R								
Reproducibility relative standard deviation								
Reproducibility limit, $r = 2,8 s_R$								

Annex B (normative)

Nutrient supply and fist test

B.1 Composition of the nutrient solution

Reagents:

Ammonium nitrate, NH_4NO_3

Calcium nitrate, $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$

Potassium nitrate, KNO_3

Mono-potassium phosphate, KH_2PO_4

Magnesium sulphate, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$

Magnesium nitrate, $\text{Mg}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$

Iron-chelate, Fe – DTPA (7% Fe)

Manganese sulfate, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$

Zinc sulphate, $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$

Borax, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$

Copper sulphate, $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$

Sodium molybdate, $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$

Nitric Acid (HNO_3), 1M

Table B.1 shows the composition of the nutrient solution.

Table B.1 — Composition of the nutrient solution

Chemical species	mmol/l	Chemical species	$\mu\text{mol/l}$
NH_4^+	1,0	Fe	15
K^+	8,0	Mn	8
Ca^+	4,0	Zn	4
Mg^{2+}	1,5	B	25
NO_3^-	16,0	Cu	0,75
SO_4^{2-}	1,25	Mo	0,5
H_2PO_4	1,5		

For preparing one litre of nutrient solution (Table B.1), the following quantities of chemicals (p.a.) have to be dissolved in water.

Table B.2 — Amounts of chemicals to be dissolved

Name	Chemical compound	mmol/l	mg/l
Ammonium nitrate	NH ₄ NO ₃	1,0	80
Calcium nitrate	Ca(NO ₃) ₂ · 4 H ₂ O	4,0	944
Potassium nitrate	KNO ₃	6,5	657
Mono-potassium phosphate	KH ₂ PO ₄	1,5	204
Magnesium sulphate	MgSO ₄ · 7 H ₂ O	1,25	308
Magnesium nitrate	Mg(NO ₃) ₂ · 6 H ₂ O	0,25	65
Name	Chemical compound	µmol/l	µg/l
Iron-chelate	Fe-DTPA (7% Fe)	15	11,97
Manganese sulphate	MnSO ₄ · H ₂ O	8	1,35
Zinc sulphate	ZnSO ₄ · 7 H ₂ O	4	1,15
Borax	Na ₂ B ₄ O ₇ · 10 H ₂ O	6,26	2,39
Copper sulphate	CuSO ₄ · 5 H ₂ O	0,75	0,19
Sodium molybdate	Na ₂ MoO ₄ · 2 H ₂ O	0,5	0,12

NOTE Stirring and warming may be necessary to dissolve the components completely.

Adjust the pH of the solution to reach 5,5 using 1M nitric acid 1.1.

All chemicals used shall be of analytical grade.

Alternatively, commercially available fertilizers of similar composition, dissolved in water can be used to provide the same concentration of prescribed elements, especially nitrogen. The pH-adjustment is necessary in any case.

The same chemicals/products should be used for both the extract test and the preparation of the fertilized peat, see 6.1.3.

B.2 Possible adjustments of the nutrient supply during the test

B.2.1 Possible reasons for nutritional adjustments

Differences in nutrient supply (especially nitrogen) can strongly affect harvested fresh weight in the pot growth test. If the nutrient content of the test sample is not allowed for the fertilization, differences in fresh weight between the test sample and the control sample can occur and in consequence of this misinterpretation about the effect of the test material on plant growth are possible. For test materials containing remarkable nutrient contents, a nutritional adjustment based on a substrate analysis is advisable, to facilitate the interpretation of differences in fresh weight. For the same reason, nitrogen adjustment (e.g. by fertigation) for test materials fixing nitrogen is possible.

B.2.2 Recommendations for nutritional adjustments

Preliminary notes:

Irrespective of the mode of nutritional adjustment always a fertilized and limed sphagnum peat (see 6.1.3) is used as control sample. Additional control samples, depending on the mode of adjustment, can be run optionally.

The nitrogen content is always based on the test sample mixed with sphagnum peat (see 6.1.2) according to Table 1.

In cases of nitrogen adjustments the supply of the prepared test sample with all other essential nutrients has to be sufficient for undisturbed plant growth.

Nitrogen content lower than $225 \text{ mg N} \cdot \text{l}^{-1}$

A water soluble complete fertilizer with essential micronutrients, is added at a rate to adjust the nitrogen level of the prepared test sample to $(225 \pm 25) \text{ mg N} \cdot \text{l}^{-1}$ (e.g. using a water soluble complete fertilizer N : P₂O₅ : K₂O – 15 : 10 : 20; see B.1).

Nitrogen content higher than $225 \text{ mg N} \cdot \text{l}^{-1}$

No nitrogen has to be added. As additional control sample a sphagnum peat (see 6.1.2) – pH-adjusted between 5,5 and 6,5 by adding limestone (see 6.1.4) – fertilized to a nutrient level similar to the prepared test sample (e.g. using a water soluble complete fertilizer N : P₂O₅ : K₂O – 15 : 10 : 20; see B.1) can be used.

Probably nitrogen-fixing test materials

Prepared test materials with less than $225 \text{ mg N} \cdot \text{l}^{-1}$ are adjusted to $(225 \pm 25) \text{ mg N} \cdot \text{l}^{-1}$ (e.g. using a water soluble complete fertilizer N : P₂O₅ : K₂O – 15 : 10 : 20; see B.1), for prepared test materials with more than $225 \text{ mg N} \cdot \text{l}^{-1}$ no nitrogen has to be added. While running out the pot growth test the prepared sample is fertigated with a nutrient solution supplying $(75 \pm 10) \text{ mg N} \cdot \text{l}^{-1}$ (e.g. $0,2 \text{ g} \cdot \text{l}^{-1}$ Ammonium nitrate; see B.1). As additional control sample a sphagnum peat (see 6.1.2) – pH-adjusted between 5,5 and 6,5 by adding limestone (see 6.1.4) – fertilized to a nutrient level similar to the prepared test sample (e.g. using a water soluble complete fertilizer N : P₂O₅ : K₂O – 15 : 10 : 20; see B.1) also fertigated with the nutrient solution can be used.

Further nutritional adjustments because of special properties of the test material always have to be done according to good horticultural practice.

B.2.3 Supplement to the report

In case of a nutritional adjustment the following information shall be added to the test report (see Clause 9):

- results of the substrate analysis including the used methods;
- nutritional adjustment (fertilization, fertigation) of the prepared test sample;
- if done, nutritional adjustment of the additional control sample;
- in case of additional control samples :
 - average germination rate and coefficient of variance of the additional control sample;
 - average inhibition of germination and coefficient of variance for the test material based on the additional control sample;
 - average fresh weight of plants per pot of the additional control sample;
 - average number of harvested plants per pot of the additional control sample;
 - average fresh weight per plant of the additional control sample;
 - difference between the average fresh weight of plants per pot of the additional control and the test sample;
 - difference between the average fresh weight per plant of the additional control and the test sample;

- average inhibition of growth and coefficient of variance for the test material based on the additional control sample;
- abnormalities in plant growth of the additional control sample.

B.3 Fist test

The fist test shall be carried out wearing flexible protective gloves.

If storage is necessary, samples are kept in accordance with EN 13040. Prior to analysis, samples are prepared according to EN 13040 (sieving < 20 mm). The material to be tested shall be adjusted to a moisture content that is suitable for plant growth prior to testing using the following procedure:

The sample is pressed in the fist. If water beads escape between the fingers, the sample is too wet. If the sample crumbles in the hand when the fist is opened, without further action, the sample is too dry. Suitable moisture content is present if the pressed sample forms an aggregate which crumbles under mild pressure, after the fist has been opened; if, on the contrary, it only deforms, it is too wet. The optimal moisture content is that which may be described as „moist as a well-squeezed sponge“. When moistening excessively dry sample material the water shall be mixed into the sample material in such a manner that it is evenly absorbed. In the case of very dry samples, this process requires thorough mixing at intervals. This procedure shall last no more than 8 h. Excessively moist samples shall be carefully air-dried (< 30 °C) and thoroughly mixed thereafter.

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