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Method of test ~ version 2.0

Plant response and weeds test for composted material

Support for the Development and Use of Standards: Compost

Method for testing plant response to composted material and its contamination by weed seeds and propagules

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WRAP helps individuals, businesses and local authorities to reduce waste and recycle more, making better use of resources and helping to tackle climate change.

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Introduction

This document sets out a method for assessing tomato plant performance and assessing contamination by weed seeds and other propagules in compost samples. Its basis is Annex D in the British Standards Institution's Publicly Available Specification for Composted Materials, 2005 edition (PAS 100:2005). The Annex D methodology was designed as a single multipurpose test assessing weed content, tomato seed germination, seedling growth and abnormalities in a sphagnum peat medium containing 20 to 33 % by volume of the compost sample under evaluation.

The improved methodology provided in this publication incorporates recommendations made as part of a 2009/10 review of the plant response test[1]. Those recommendations included retention of the multipurpose Annex D test, so long as it could be made clearer and more robust. This document seeks to address those recommendations.

This updated methodology aims to clarify the scope of the test and the materials to be used. It clarifies the quantities of peat and compost to use, the minimum requirements for light intensity and duration, and provides more detailed instructions for assessing, calculating and reporting the test results. The methodology also requires randomisation of treatment replicates and includes instructions for assessing and reporting the validity of the test each time it is performed.

At the time of publication there are no laboratory repeatability and reproducibility data available for the updated methodology instructed in this document. The means by which such data could be obtained and evaluated will be considered by the Renewable Energy Assurance Ltd (REAL)). As part of its Compost Certification Scheme, REAL will monitor the performance of each laboratory that it approves to carry out this methodology.

Since the introduction of the original plant response and weeds test, incidences of herbicide contamination in compost have been reported outside the UK (for example, the USA and New Zealand). In this context, WRAP has carried out a number of investigations and concluded that the risk of harm from contaminated composts in the UK is low and that the tomato plant response test is suitable for screening composts intended for use in agriculture and field horticulture. However, the evidence suggests that the tomato performance test is not sufficiently sensitive to detect herbicide concentrations of interest to the growing media sector, specifically when composts are to be included in growing media used for cultivating sensitive plant species.

On this basis, a more sensitive plant response test has been developed, using field bean (*Vicia faba* cv Fuego), which is recommended for testing composts destined for use as a growing media. Since growing media could be used to cultivate both sensitive and insensitive plant species, it is recommended that all batches of compost intended for supply to the growing media sector be subjected to testing using the field bean.

The methodology for carrying out the field bean plant response test is described in the Annex A to this document.

[1] *Review of the plant growth and weed propagule tests used in the PAS100 specification for quality compost*. WRAP project reference OFW006-002.

Contents

1.0 Principle

This method of test investigates how a compost-amended peat growing medium (the test sample) affects tomato seed germination, top-growth and health, when compared with a standard peat growing medium (the control) under controlled conditions over a 28 day test period. The effect of variable soluble salt contents in the composts under test (as judged by their electrical conductivity) is substantially limited by controlled dilution of the compost with sphagnum peat that has low conductivity. This means that any significant negative response in the test sample that is not seen in the control can be taken to indicate phytotoxic factors in the compost, which might then require further testing before the compost can be used.

This method includes simultaneous assessment of weed seeds and other propagules that may be present in the test sample, taking account of any that appear in the control.

This test is designed to assess many of the compost grades used in growing media and most soil improvers, such as those with particle size ranges 0 - 6 mm, 0 - 10 mm, 0 - 12 mm, 0 - 15 mm, 0 - 20 mm, 0 - 25 mm, and 0 - 40 mm. However, is not suitable for composts that retain no soil-like particles, such as mulch grades that consist only of coarse woody particles. PAS 100 does not require such mulch grades to undergo this test for tomato plant performance and weed seeds.

The person who carries out each test should be experienced in carrying out plant germination, growth and weed seed/propagule tests or supervised or trained by such a person.

Please note that the test sample (comprising a mix of compost and peat, with inorganic fertiliser and dolomitic limestone) is not intended to represent an optimized commercial growing media formulation.

2.0 Apparatus

2.1 Seed trays

Seed trays, nominal size 210 mm long, 150 mm wide and 50 mm deep. The capacity of each chosen tray when filled shall be 1.5 litres, to accommodate the requirements of Section 5.1 (below).

2.2 Capillary matting and water

Capillary matting, for use with water fit for drinking by humans, or preferably water of Grade 3 according to BS EN ISO 3696.

NOTE Capillary matting helps maintain adequate moisture in the PBGM control and test sample materials during the course of the 28 day test. It should be replaced at least once in every two months. Inadequate moisture will adversely affect tomato seedling germination and growth.

2.3 Controlled environment for plant growth

Greenhouse, plant growth chamber or other controlled environment for plant growth, with adequate artificial lighting (see Section 2.11), capable of being maintained in the temperature range 20 °C to 25 °C.

2.4 Transparent plastic covers

Transparent plastic covers, shaped and sized to cover individual seed trays.

2.5 Sphagnum peat

Sphagnum peat, medium grades e.g. H4/H5 on the von Post scale with electrical conductivity of $\leq$ 100 µS cm^{-1}.

NOTE Peat should be lightly damp and friable. Extra time may be necessary to re-wet the peat before the start of the test, using water that meets the specification in Section 2.2.

2.6 Seed template and board

Seed template and board, prepared from a rigid material (e.g. plywood) to fit the seed tray exactly when filled, providing a guide that allows ten tomato seeds to be evenly distributed in each seed tray. Drill 2 rows of holes (5 holes per row, each approximately 5 mm diameter) in the template at approximately 35 mm intervals. A sturdy handle should be affixed to the template to enable its use also as a tamping/levelling device.

An acceptable alternative is a rigid board with a sturdy handle, that fits the seed tray exactly when filled and has ten protrusions (e.g. wooden pegs or screws) firmly attached and evenly distributed as instructed above. The length of each peg shall be 6 mm and width approximately 5 mm.

2.7 Balances

Balance one; capacity 200 g, accurate to 0.01 g or better. Balance two; capacity 3 kg, accurate to 1 g.

2.8 Fertiliser

A complete powdered, inorganic NPK fertiliser containing trace elements that is recommended for the formulation of growing media. The N to K ratio of the selected fertiliser should be circa 1:1[2].

2.9 Ground dolomitic limestone

Ground dolomitic (magnesian) limestone, fine horticulture grade.

2.10 Tomato seeds

Viable tomato seeds, cultivar Shirley F1 hybrid.

NOTE See Section 10.2 for guidelines on tomato seed storage, which affects the viability of seeds.

2.11 Artificial lighting and light meter

Professional horticultural specification lamps, for supporting plant growth when natural light is at low levels, placed and used such that the minimum light intensity at plant level (as determined by light meter) is maintained at 6000 lux at all times during each 16 hour day (light) period.

NOTE In the countries of the United Kingdom, 'low' natural daylight tends to occur from mid October to mid March even under good glasshouse conditions. Examples of potentially suitable lamp types are high pressure sodium, fluorescent or metal halide.

2.12 Sieve

Sieve, with 10 mm apertures formed by square mesh.

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[2] A suitable example is Horti-base mix D, a base fertiliser for peat media; 15:10:16). See http://www.hortifeeds.co.uk/select_frame_set.html for details of the exact formulation. Horti-Base is a trade mark owned by Hortifeeds Park Farm, Kettlethorpe, Lincoln LN1 2LD, United Kingdom, and is the trade name of a product supplied by Hortifeeds.

PG Mix™ 14-16-18 is an example of another common formulation that could be used. See http://www.frontierag.co.uk/frontier/pages/page.jhtml?page_id=14700081&section_name=100003 for details.

The product examples are given for the convenience of users of this specification and do not constitute an endorsement of the product named by any organisation referred to in this document. Equivalent products may be used if they lead to the same results.

3.0 Preparation of peat-based growing medium (PBGM)

3.1 Sieving peat

Pass sufficient peat (Section 2.5) through the sieve (Section 2.12) to obtain at least 8.5 litres of sieved peat (< 10 mm particles).

3.2 Electrical conductivity and bulk density of sieved peat

Determine the electrical conductivity of the sieved peat (report item B in Section 9.1) in accordance with BS EN 13038. This requires determination of its laboratory compacted bulk density (LCBD) (report item C in Section 9.1), in accordance with BS EN 13040.

3.3 Adding limestone and fertiliser to the sieved peat

Prepare the PBGM by mixing both 18 (± 0.05) g of dolomitic limestone (Section 2.9) into 4.5 litres of sieved peat and sufficient fertiliser (Section 2.8) to provide 150 mg N / l[3]. Record the mass of the 4.5 litres of sieved peat (report item D in Section 9.1). Record the mass (g) of dolomitic limestone (report item E in Section 9.1) and mass (g) of fertiliser (report item F in Section 9.1) mixed into the sieved peat to form the PBGM control.

The LCBD (laboratory compacted bulk density) values used shall correspond with the sieved peat and sieved compost sample used to form the PBGM and test sample. Moisture levels should be unaltered between the time when their LCBDs were determined and the time when the PBGM control and test samples are set up (see Section 5.1). If the moisture level of either material becomes different from when the LCBD was measured, a further determination of LCBD shall be undertaken and that value used.

Thorough mixing of components is essential. This can be achieved by first adding dolomitic limestone and fertiliser to a small volume of sieved peat (0.5 litres to 1.0 litres) then thoroughly mixing these components together in a suitable container. Spread the remaining sieved peat thinly on a flat surface and evenly distribute the lime and fertiliser mix over it. Mix thoroughly using techniques such as coning and quartering to achieve even distribution of lime and fertiliser throughout the sieved peat.

[3] *For example, if 15:10:16 NPK fertiliser is used, this would require addition of 1g fertiliser per litre of sieved peat*

4.0 Preparation of the test sample (TS)

4.1 Compost sample preparation, including sieving

Prepare the compost sample, 'as received' by the laboratory, in accordance with BS EN 13040, with particular reference to its clause 8.1.

NOTE The compost sample should be representative of the batch of compost under test, and have been obtained by following the instructions in BS EN 12579.

Pass sufficient compost through the sieve (Section 2.12) to obtain at least 2 litres of sieved compost (< 10 mm particles). Separately retain the fractions with particle sizes greater and less than 10 mm. Record the mass of each of these fractions then calculate and record the percentage of compost particles less than 10 mm in the compost 'as received' (report item H in Section 9.1).

4.2 Electrical conductivity and bulk density of sieved compost

Determine the electrical conductivity of the sieved compost (<10mm particles) (report item I in Section 9.1) in accordance with BS EN 13038. This requires determination of its laboratory compacted bulk density (LCBD) (report item J in Section 9.1), in accordance with BS EN 13040.

4.3 Ratio of peat to compost in the test sample

Use Equation 1 to calculate the number of parts of sieved peat (Y) to add to 1 part of sieved compost (< 10 mm particles) to produce a test sample with the ideal electrical conductivity of 300 µS cm^{-1}. However, for some composts with electrical conductivities that are either very low or very high, using Equation 1 may result in the addition of very little peat or else the addition of excessive amounts of peat to produce test samples with the preferred electrical conductivity of 300 µS cm^{-1}. Hence, to ensure that acceptable amounts of both peat and compost are present within the test sample, it is important to note that there is a minimum and a maximum proportion of sieved peat that sieved compost should receive.

If Y is:

- < 2, use no less than 2 parts sieved peat to 1 part sieved compost (in this case conductivity of the test sample will be < 300 µS cm^{-1}); or
- > 4, use no more than 4 parts sieved peat to 1 part sieved compost (in this case conductivity of the test sample will be > 300 µS cm^{-1}).

The peat:compost ratios for all test samples should be within the range 2:1 and 4:1 at all times.

Equation 1
Y = (sieved compost electrical conductivity – 300) ÷ (300 – sieved peat electrical conductivity)
NOTES *1 Y = parts of sieved peat (Section 3.1) added to one part of sieved compost (Section 4.2), by volume.* *2 Report Y to two decimal places (report item K in Section 9.1).* 3 *Units of electrical conductivity are expressed in µS cm^{-1}.*

4.4 Compost mixed with peat for the test sample

At least 4.5 litres of test sample is required, consisting of sieved peat (Section 3.1) thoroughly mixed with sieved compost (Section 4.1) to achieve the ratio required in Section 4.3. Determination of quantities is most simply achieved by weighing sieved peat or compost, after the required volume of each has been calculated.

Example calculations of volumes of peat and compost required:
Y = 2.00 parts peat added to 1.00 part compost, by volume.
Volume of peat required = 4.50 ÷ (2.00 parts peat + 1.00 part compost), which is 3.00 litres.
Volume of compost required = 4.50 minus 3.00, which is 1.50 litres.

Calculate the number of grammes of each sieved material required to be mixed together to form 4.5 litres of test sample. Do this by multiplying the required volume (in litres) of each material by its laboratory compacted bulk density (grammes/litre). Record the grammes of sieved peat (report item L in Section 9.1) and sieved compost (report item M in Section 9.1) used in the test sample.

Example calculations of grammes of peat and compost required for a test mix of 2.00 parts peat to 1.00 part compost, by volume:
If 3.00 litres peat is required and its LCBD is 200g/l, weigh out 3.00 x 200 = 600 g.
If 1.50 litres compost is required and its LCBD is 800 g/l, weigh out 1.50 x 800 = 1,200 g.

The LCBD values used shall correspond with those of the peat and compost sample used to form the PBGM and test sample. The moisture levels of the materials used to make up the test sample and control should be the same as when their LCBDs were determined. If this is not the case, a further determination of LCBD shall be undertaken and that value used.

After weighing out the appropriate grammes of sieved peat and compost, thoroughly mix these materials together.

4.5 Adding limestone and fertiliser to the test sample

Mix dolomitic limestone (Section 2.9) into the test sample at the rate of 4 (± 0.05) g per litre of peat used, and record the grammes of dolomitic limestone used (report item N in Section 9.1).

Mix into the test sample sufficient fertiliser (Section 2.8) to provide 150 mg water-soluble N / l in the whole text mix, supplied specifically by the fertiliser. Record the grammes of fertiliser mixed into the test sample (report item O in Section 9.1).

NOTE Addition of fertiliser should achieve a moderate amount of water-soluble nutrients in the test mix, irrespective of water-soluble nutrients released from the compost. The latter are not determined in this test.

Thorough mixing of components is essential. This can be achieved by first adding lime and fertiliser to a small volume of substrate (0.5 litres to 1.0 litre) then thoroughly mixing these components together in a suitable container. Spread the remaining substrate thinly on a flat surface and evenly distribute the lime and fertiliser mix over the remaining substrate. Mix thoroughly using techniques such as coning and quartering to achieve even distribution of lime and fertiliser throughout the substrate.

5.0 Procedures for setting up, monitoring and recording the responses of tomato seeds and any weed seeds or propagules

5.1 Introduction: set up of PBGM control and test sample

The PBGM control shall be set up in 3 trays (Section 2.1), representing one set of PBGM control. The compost plus peat test sample shall be set up in 3 trays, as one set of test sample. One set of PBGM control (3 trays) is allowed for up to 6 sets of test sample (18 test sample trays, for 6 different compost samples), provided that all are set up on the same day. Record test set up such that each set of PBGM control trays can be readily associated with the set(s) of test samples allocated to it.

NOTE This applies when the laboratory has capacity to run tests on more than 21 trays at any one time, or at a lower level of capacity if one set of PBGM control is used for less than 6 sets of test sample.

5.1.1 Setting up PBGM control and test sample using a seed template with holes

Put 1.3 litres of PBGM (prepared as instructed in Section 3) into each of 3 trays (Section 2.1) to act as one set of PBGM control. Put 1.3 litres of TS (prepared as instructed in Section 4) into each of 3 trays (Section 2.1) to act as one set of test sample. Lightly firm the material in each tray by pressing into corners and around the edges with finger tips. Tamp and level the surface of the growing medium using the template/board (Section 2.6) with light pressure. Separately retain sufficient of the PBGM and test sample materials for later covering of the positioned tomato seeds.

Use a fine rose watering can to water (Section 2.2) all six trays until thoroughly moistened. Avoid over-watering as this leaches nutrients from the growing media.

NOTE Watering before adding the growing medium layer that covers the seeds avoids the risk that they are displaced when watering.

For each of the trays in turn, place the seed template (Section 2.6) on the surface of the growing medium. Place one tomato seed (Section 2.10) per hole then remove the seed template. Cover the seeds in PBGM trays by sieving (Section 2.12) a layer of PBGM over the surface, such that the layer is 6 mm deep and each tray then contains 1.5 litres of PBGM. Cover the seeds in the test sample (TS) trays by sieving (Section 2.12) a layer of test sample over the surface, such that the layer is 6 mm deep and each tray then contains 1.5 litres of test sample.

NOTE A layer of six millimetres of suitable material covering the seeds assists shedding of the seed coat soon after emergence, which indirectly facilitates later expansion of the cotyledons (seed leaves).

5.1.2 Setting up PBGM control and test sample using a seed template with protrusions

The instructions in this sub-section provide an alternative to those in 5.1.1.

Put 1.5 litres of PBGM (prepared as instructed in Section 3) into each of 3 trays (Section 2.1) to act as the control. Put 1.5 litres of TS (prepared as instructed in Section 4) into each of 3 trays (Section 2.1) to act as the test sample. Lightly firm the material in each tray by pressing into corners and around the edges with finger tips. Tamp and level the surface of the material using the template/board (Section 2.6) with light pressure.

For each of the trays in turn, place the seed template (Section 2.6) on the surface of the growing medium such that its pegs make holes in the growing medium, then carefully remove the template. Put one tomato seed (Section 2.10) into each hole then lightly press the growing medium together to close the hole at its surface.

Use a fine rose watering can to water (Section 2.2) all six trays until thoroughly moistened. Take care that water flow during watering does not displace the tomato plant seeds. Avoid over-watering as this leaches nutrients from the growing media.

5.1.3 Covering and placing out trays

After setting up the growing media and tomato seeds in the control and test sample trays and watering, cover the trays with individual transparent plastic covers (Section 2.4) then place on moist capillary matting (Section 2.2) in a greenhouse, plant growth chamber or other controlled environment for plant growth (Section 2.3). Subject to good control of growing conditions as specified in this methodology, the covers do not have to be used during summer months and in any better than average seasonal weather periods (see note) during spring or autumn. If they are not used or removed early (see Section 5.2) records must reflect this.

NOTE When using transparent plastic covers, over-heating of the seeds / seedlings can occur when ambient temperature is above 25°C or when natural daylight intensity is very high and ambient temperature is above 20°C. The temperatures stated are guidelines, and the person responsible for the test should use their judgement when deciding whether to use transparent plastic covers, including during spring or autumn.

Place all trays in a randomised block design.

NOTE This avoids uneven impact of any corner and edge-effects in terms of temperature, light intensity and space in which tomato plants can grow. Careful consideration should be given to the positioning of artificial lighting, both horizontally and vertically, and the direction from which natural light reaches the plants. Spacing of trays should be sufficient to prevent shading effects from plants growing in adjacent trays.

5.1.4 Checking light intensity

After each tray has been placed in its test location use a light meter to check whether light intensity at the surface of the growing media is at or above 6000 lux. Adjustments to artificial lighting shall be made until at least 6000 lux at the surface of the growing media is achieved. If this requirement is not met, the test is invalid.

5.1.5 Checking controlled environment temperature

After each PBGM control and TS tray has been placed in its test location check whether the temperate of the controlled environment for plant growth is maintained in the temperature range 20 to 25 °C for at least 16 hours per day, including the hours of daylight. If these criteria are not met and maintained the test is invalid.

5.1.6 Inspections and maintenance of a suitable controlled environment

Inspect all trays daily. Remove the transparent plastic cover from its tray when 5 of the 10 seeds sown have emerged through the surface of the growing medium. Practitioners should note the differences between emerged seedlings (see Figure 1) and germinated seedlings (see Figure 2 and definition in next paragraph).

Figure 1. Emerged tomato plant seedlings

At 14 days after sowing (or sooner if all seeds sown in the PBGM control and test sample trays have germinated), the frequency of inspection can be reduced. However, continued daily inspections are encouraged and shall be frequent enough to ensure that adequate moisture in the growing media is maintained.

'Germinated' is defined as the stage when the cotyledons (seed leaves) have expanded and all visible parts of the seedling are 'normal' or 'usable' for horticultural purposes and describable as 'ready to prick out' (see Figure 2). Seedlings should reach this stage approximately 10 days after sowing.

Figure 2. Germinated tomato plant seedlings

Maintain adequate moisture content within the growing media by watering whenever necessary using water as specified in Section 2.2. Use a fine rose for watering until the point at which all seedlings that have germinated have expanded their true leaves (see Figure 3). After this point a rose with larger holes may be used, but care should still be taken not to disturb or dislodge plants during watering. Take particular care not to over-water.

NOTE 1 An example of particularly poor practice is watering to the point at which water drains through the bottom of the seed trays.

NOTE 2 True leaves are those that develop after the 2 cotyledons (seed leaves). True leaves are also described as the first hardy leaves, usually the second pair, on a new plant.

Figure 3. Tomato plant seedlings with their true leaves expanded

Maintain the controlled environment for plant growth in the temperature range 20 °C to 25 °C for at least 16 hours per day. A minimum temperature of 10 °C shall be maintained for the remaining hours per day. Check and record the temperature on each day of the test. Take corrective action if temperature moves outside this range. Record the corrective action and its effect on temperature within the controlled environment.

NOTE It is recommended that a minimum temperature of 20 °C be maintained for 24 hours per day, but acknowledged that this may not be cost effective for all practitioners of this test.

At a height equivalent to growing medium surface level, maintain light intensity at a minimum of 6000 lux throughout each 16 hour day (light) period. Use a light meter to check this at test set up and then daily, when any natural light is at its weakest during the 16 hour day (light) period.

If light intensity at the height equivalent to growing medium surface level is less than 6000 lux, adjust the artificial lighting (Section 2.11) then recheck the light intensity. Record the results, in lux, of each daily light meter check and any recheck. Record also the number of hours during each day of the test when the artificial lighting was on.

NOTE It is likely that artificial lighting will have to be switched on for 16 hours each day of the test from November to March inclusive. During September, October and April it is likely that artificial lighting will have to be switched on during early and late daylight hours when natural light intensity could be less than 6000 lux at the surface of the growing media.

5.2 Recording observations

5.2.1 Tomato plant germination

At 10 days after sowing, for each tray of PBGM control record the total number of germinated tomato seedlings (report item Q in Section 9.1) and for each tray of test sample record the total number of germinated tomato seedlings (report item R in Section 9.1). See definition of 'germinated' in Section 5.2 and photo in Figure 2. Do not record seedling emergence from the PBGM control or test sample growing medium as 'germinated' (see Figure 1 in Section 5.2).

NOTE This applies only to the tomato plants that germinated and grew from the deliberately sown tomato seeds. Any other tomato seed that germinated is a weed, so is excluded from the tomato plant germination results.

At 14 days after sowing, remove any seedlings that have failed to develop normally, i.e. those which break through the PBGM control or test sample material's surface but do not otherwise thrive, and any seedlings whose cotyledons are very small or shrivelled. Do not include any such seedlings in the recording, calculation and reporting of the number of germinated tomato seeds per tray of PBGM control and per tray of test sample at 14 days after sowing. Where the cotyledons have grown to full size but are still held together by the seed coat, it is acceptable to carefully pinch or snip off the seed coat from the tip of the cotyledons and record these seedlings as 'germinated'.

At 14 and 28 days after sowing, for each tray of PBGM control record the total number of germinated tomato seedlings (report item Q in Section 9.1) and for each tray of test sample record the total number of germinated tomato seedlings (report item R in Section 9.1).

5.2.2 Tomato plant growth and abnormalities

At 14 and 28 days after sowing, compare and record any visible differences in tomato plant growth characteristics between those that grown in the 3 TS trays and those that have grown in the 3 PBGM control trays. Note any abnormal symptoms in plants that have grown in the test sample or PBGM control trays, and take digital photographs (report item U in Section 9.1). Check for and record whether there are any abnormalities in any test sample-grown plants, specifically any **not** observed in any of the PBGM control-grown plants (report item V in 9.1).

Examples of abnormalities are: Evidence of surface rooting, plant distortions, unusual colouring, lesions, chlorosis (yellowing) or other abnormalities in shoots or leaves. Any incidence of 'purpling' in tomato plant seedlings is likely to be caused by temperatures significantly lower than the minimum specified for this test rather than of nutrient deficiency, contamination or some other problem.

NOTE The recording of abnormalities is important as they can indicate the presence or absence of phytotoxins (such as volatile organic acids, pesticides or herbicides) which may not be apparent from the germination and mean fresh mass per tomato plant results. See Section 6.2 for further instructions relating to abnormalities in test sample-grown plants and Section 7.2 in respect of abnormalities in PBGM control-grown plants.

5.2.3 Recording tomato plant masses

At 28 days after sowing, cut the tomato plants off at the surface of the growing medium in each PBGM control tray and immediately record the fresh mass (± 0.01 g) of tomato plant 'top growth' (TgFM) from each tray (report item X in Section 9.1). Do the same with the tomato plants that have grown in each of the test sample trays (report item Y in Section 9.1).

NOTE This applies only to the tomato plants that germinated and grew from the deliberately sown tomato seeds. Any other tomato seed that germinated is a weed, so is excluded from the TgFM results.

5.2.4 Weed seeds and propagules

At 28 days after sowing, record the number of weed seeds or propagules that have germinated in each of the 3 PBGM control trays (report item DD in Section 9.1) and in each of the 3 test sample trays (report item EE in Section 9.1). Any tomato plant that has germinated in addition to those sown shall be recorded and reported as a weed.

6.0 Calculation and expression of tomato plant results

6.1 Tomato plant germination

For each of the periods 10, 14 and 28 days after sowing, calculate the total number of germinated tomato plants in the 3 test sample (TS) trays as a percentage of the total number of germinated tomato plants in the 3 PBGM control trays (report item S in Section 9.1).

In the calculations below, PBGM control trays are abbreviated as P1, P2 and P3, and test sample trays are abbreviated as T1, T2 and T3:

At 10 days after sowing, the number of germinated tomato plant seeds in T1 plus T2 plus T3, divided by number of germinated tomato plants in P1 plus P2 plus P3, multiplied by 100.

At 14 days after sowing, the number of germinated tomato plants in T1 plus T2 plus T3, divided by number of germinated tomato plant seeds in P1 plus P2 plus P3, multiplied by 100.

At 28 days after sowing, the number of germinated tomato plants in T1 plus T2 plus T3, divided by number of germinated tomato plants in P1 plus P2 plus P3, multiplied by 100.

6.2 Abnormalities

Digital photographs of all abnormalities in plants shall be included in, or provided in conjunction with, the report of test results (item U in Section 9.1).

Check observation records and any digital photographs taken for abnormalities in any of the tomato plants grown in the 3 test sample trays that were **not** observed in any of the tomato plants grown in the 3 PBGM control trays. The absence or presence of such abnormalities shall be reported (item V in Section 9.1).

NOTE See 7.2 for instructions on assessing the validity of the test according to any abnormalities in the PBGM control-grown plants only.

6.3 Tomato plant mass

All data used in the calculations below shall be the '28 days after sowing' values, as appropriate to tomato plant germination or TgFM. PBGM control trays are abbreviated as P1, P2 and P3. Test sample trays are abbreviated as T1, T2 and T3.

Using the TgFM results recorded as instructed in Section 5.2.3, calculate the mean TgFM (± 0.01 g) per tomato plant that grew in each the 3 trays of PBGM control (report item Z in Section 9.1) and each of the 3 trays of test sample (report item AA in Section 9.1), at 28 days after sowing.

Calculation of report item Z in Section 9.1:

Mean TgFM per tomato plant for all 3 PBGM control trays (in grammes) =

(total TgFM of tomato plants in P1 + total TgFM of tomato plants in P2 + total TgFM of tomato plants in P3)

÷

(number of germinated tomato plants in P1 + number of germinated tomato plants in P2 + number of germinated tomato plants in P3)

Calculation of report item AA in Section 9.1:

Mean TgFM per tomato plant for all 3 test sample trays (in grammes) =

(total TgFM of tomato plants in T1 + total TgFM of tomato plants in T2 + total TgFM of tomato plants in T3)

÷

(number of germinated tomato plants in T1 + number of germinated tomato plants T2 + number of germinated tomato plants T3)

Using results for report items Z and AA, calculate the mean TgFM (± 0.01 g) per tomato plant for all 3 test sample trays as a percentage of the mean TgFM per tomato plant for all 3 PBGM control trays, at 28 days after sowing and referred to in the calculation below as result C (report item BB in 9.1).

Calculation of report item BB in Section 9.1:

Mean TgFM per tomato plant for all 3 test sample trays as a percentage (to 2 decimal places) of the mean TgFM per tomato plant for all 3 PBGM control trays, at 28 days after sowing =

(Result AA ÷ Result Z) x 100

6.4 True leaves

N.B. The instructions in sub-section 6.4 are recommendations, not requirements. *They are intended to provide useful information to help evaluate test performance and improve the methodology in the future. However, due to cost implications and introduction of the test validity criteria in Section 7.0, the recording, evaluation and reporting of data on true leaves is not obligatory.*

At 28 days after sowing, record the total number of true leaves on all tomato plants that grew in each tray of PBGM control (report item GG in Section 9.1). At 28 days after sowing, record the total number of true leaves on all tomato plants that grew in each tray of test sample (report item HH in Section 9.1).

NOTE True leaves are the leaves of a seedling that develop after the cotyledons (see note to Section 5.2). They are also described as the first hardy leaves, usually the second pair, on a new plant (see Figure 3 in Section 5.2).

Calculate the mean number of true leaves per tomato plant that grew in each of the 3 PBGM control trays (report item II in Section 9.1).

Calculate the mean number of true leaves per tomato plant that grew in each of the 3 test sample trays (report item JJ in Section 9.1).

NOTE Depending on the time of year and control of the growing environment, tomato plants that grow in the PBGM control trays should achieve at least 3 to 4 true leaves per plant.

7.0 Validity of the test

NOTE Results of the test are influenced by the suitability of the test environment, its control to suit the germination and growth of tomato plants and the viability of tomato seeds used. The criteria in this section of the methodology set out the minimum PBGM control performance required for the test to be valid.

7.1 Tomato plant germination

Report the total number of tomato seeds that germinated in all 3 PBGM control trays at 14 days after sowing (item T in Section 9.1).

PBGM control trays are abbreviated as P1, P2 and P3.

If fewer than 27 tomato seedlings have germinated in all 3 PBGM control trays by 14 days after sowing, the test is invalid. Report this result and declare that the test is invalid if it is less than 27 or valid if it is equal to or greater than 27 (report item T in Section 9.1).

7.2 Abnormalities

Digital photographs of abnormalities shall be included in the report of test results or provided in conjunction with it (report item U in Section 9.1).

Check observation records and any digital photographs taken for abnormalities in any of the tomato plants grown in the 3 PBGM control trays, and report their absence or presence (report item W in Section 9.1).

The presence of any abnormality in any plant that grew in any of the 3 PBGM control trays shall make the test invalid. Declare that the test is valid if there were no abnormalities in the PBGM control-grown plants or invalid if any abnormalities were present in the PBGM control-grown plants (see item W in Section 9.1).

7.3 Tomato plant mass

Calculate the mean top growth fresh mass (TgFM) per tomato plant for all 3 PBGM control trays, at 28 days after sowing (report item CC in Section 9.1).

PBGM control trays are abbreviated as P1, P2 and P3.

> Calculation: At 28 days after sowing, the total TgFM (± 0.01 g) of tomato plants in P1 plus P2 plus P3, divided by the total number of germinated tomato plants in those trays. Express the result in grammes, to 2 decimal places.

If the mean TgFM per tomato plant for all 3 PBGM control trays at 28 days after sowing is less than 1.5 g the test is invalid. Report this result and declare that the test is invalid if it is less than 1.5 g or valid if it is equal to or greater than 1.5 g (report item CC in Section 9.1).

NOTE The minimum figure specified is low, corresponding with the least that may be achieved when the test is carried out during November, December, January, February or March. It is anticipated that the minimum figure will be adjusted after data from laboratories that have used this version of the methodology has been collated and evaluated.

8.0 Calculation and expression of weeds result

8.1 Weed plants per litre of compost 'as received'

Calculate and report the number of weed plants per litre of compost 'as received', that emerged over the 28 days of the test (item FF in Section 9.1). The calculation should be undertaken as follows:

$$\left\{\frac{(WT1+WT2+WT3)-\left(TgPT\times\left(\frac{WP1+WP2+WP3}{TgPP}\right)\right)}{\left(\frac{TgCT}{\left(\frac{P\%CAR}{100}\right)}\right)}\right\}\times LCBDC$$

Where:

LCBDC = Laboratory compacted bulk density of sieved compost (< 10 mm particles)
P%CAR = Percentage of < 10 mm particles in compost 'as received'
TgCT = Total grammes of compost in test sample trays (set of 3)
TgPP = Total grammes of peat in PBGM control trays (set of 3)
TgPT = Total grammes of peat in test sample trays (set of 3)
WT1 = Number of weed plants in test sample tray 1
WT2 = Number of weed plants in test sample tray 2
WT3 = Number of weed plants in test sample tray 3
WP1 = Number of weed plants in PBGM control tray 1
WP2 = Number of weed plants in PBGM control tray 2
WP3 = Number of weed plants in PBGM control tray 3

9.0 Report of test results

9.1 Test information and results required in the report

The test report shall include the following information:

A. a reference to this method;

Materials used in the PBGM control

B. the electrical conductivity of the sieved peat (< 10 mm particles, see Section 3.2);
C. the laboratory compacted bulk density of the sieved peat (< 10 mm particles, see Section 3.2);
D. the mass (g) of sieved peat used to form the PBGM control (see Section 3.3);
E. the mass (g) of dolomitic limestone mixed in to form the PBGM control (see Section 3.3);
F. the mass (g) of fertiliser mixed in to form the PBGM control (see Section 3.3);

Materials used in the test sample

G. compost sample identification;
H. the % mass/mass of < 10 mm particles in the compost as received (see Section 4.1);
I. the electrical conductivity of sieved compost (< 10 mm particles, see Section 4.2);
J. the laboratory compacted bulk density of sieved compost (< 10 mm particles, see Section 4.2);
K. the number of parts of sieved peat (by volume, to 2 decimal places) added to one part of the sieved compost (by volume, to 2 decimal places) to form the test sample (see Section 4.3, e.g. 2.00 peat : 1.00 compost);
L. the mass (g) of sieved peat used in the test sample (see Section 4.4);
M. the mass (g) of sieved compost used in the test sample (see Section 4.4);
N. the mass (g) of dolomitic limestone mixed in to form the test sample (see Section 4.5);
O. the mass (g) of fertiliser mixed in to form the test sample (see Section 4.5);

Factors affecting the test

P. any factors known or suspected to have affected the test and its validity (see Sections 5.1.4, 5.1.5 and instructions in Section 5.2 about checks on temperature and light intensity);

Tomato plant germination

Q. the number of germinated tomato plants in each of the 3 trays of PBGM control at 10, 14 and 28 days after sowing (see Section 5.2.1);
R. the number of germinated tomato plants in each of the 3 trays of test sample at 10, 14 and 28 days after sowing (see Section 5.2.1);
S. the total number of germinated tomato plants in all 3 test sample trays expressed as a percentage of the total number of germinated tomato plants in all 3 PBGM control trays, at 10, 14 and 28 days after sowing (see Section 6.1);
T. the number of tomato seeds that germinated in all 3 PBGM control trays at 14 days after sowing, and declaration that the test is invalid if fewer than 27 OR is valid if equal to or more than 27 (see Section 7.1);

Abnormalities

U. digital photographs of all plant abnormalities (see Section 5.2.2, these may be provided in conjunction with the report of test results if they cannot be readily incorporated within the report);
V. declaration of the absence or presence of abnormalities in tomato plants grown in any of the 3 test sample trays that were not present in plants grown in any of the 3 PBGM control trays (see Section 6.2);
W. declaration of the absence or presence of abnormalities in tomato plants grown in any of the 3 PBGM control trays, and declaration that the test is valid if such abnormalities were absent or invalid if they were present (see Section 7.2);

Tomato plant mass

X. the total top growth fresh mass (± 0.01 g) of tomato plants that grew in each of the 3 trays of PBGM control at 28 days after sowing (see Section 5.2.3);
Y. the total top growth fresh mass (± 0.01 g) of tomato plants that grew in each of the 3 trays of test sample at 28 days after sowing (see Section 5.2.3);
Z. the mean top growth fresh mass (± 0.01 g) per tomato plant that grew in each the 3 trays of PBGM control, at 28 days after sowing (see Section 6.3);
AA. the mean top growth fresh mass (± 0.01 g) per tomato plant that grew in each the 3 trays of test sample, at 28 days after sowing (see Section 6.3);
BB. the mean top growth fresh mass (± 0.01 g) per tomato plant for all 3 test sample trays as a percentage (to 2 decimal places) of the mean top growth fresh mass per tomato plant for all 3 PBGM control trays, at 28 days after sowing (see Section 6.3);
CC. the mean top growth fresh mass per tomato plant (± 0.01 g) for all 3 PBGM control trays at 28 days after sowing, and declaration that the mean mass per tomato plant part of the test is invalid if the calculated mean is less than 1.5 g per tomato plant OR is valid if more than or equal to 1.5 g per tomato plant (see Section 7.3);

Weed seeds / propagules

DD. the number of weed plants in each of the 3 trays of PGBM control (see Section 5.2.4);
EE. the number of weed plants in each of the 3 trays of test sample (see Section 5.2.4); and
FF. the number of weed plants per litre of compost 'as received', that emerged over the 28 days of the test (see Section 8.1, express result to 1 decimal place).

NOTE to item FF: The result is unlikely to be a whole number because it takes into account the litres of compost used in 3 test sample trays and the proportion of particles larger than 10 mm in the compost 'as received' (before being sieved).

9.2 Test results optional in the report

The following information is optional for inclusion in the test report:

Tomato plant true leaves

GG. the total number of true leaves on all tomato plants that grew in each of the 3 PBGM control trays at 28 days after sowing (see Section 6.4);
HH. the total number of true leaves on all tomato plants that grew in each of the 3 test sample trays at 28 days after sowing (see Section 6.4);
II. the mean number of true leaves per tomato plant that grew in each of the 3 PBGM control trays at 28 days after sowing (see Section 6.4); and
JJ. the mean number of true leaves per tomato plant that grew in each of the 3 test sample trays at 28 days after sowing (see Section 6.4).

9.3 REAL's reporting requirements for its Approved Laboratories

Laboratories approved by the REAL for testing samples of compost under its Compost Certification Scheme are required to report test results using REAL's template or ensure that their reporting system generates reports set out in the same way, subject to REAL's check on how the system performs the calculations.

In terms of the plant response and weed seeds and propagules test, the report template supplied by REAL includes a few parameters additional to those required in Section 9.1.

10.0 References and tomato seed storage

10.1 Normative references

The most recent editions of each of these standards shall be used:

BS EN ISO 3696, Water for analytical laboratory use - Specification and test methods.

BS EN 12579, Soil improvers and growing media – Sampling.

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

BS 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.

10.2 Tomato seed storage

Storage temperature, relative humidity and seed moisture are the important factors that determine how long seed can be stored without loss of germination (viability). In general, longer seed storage life is obtained when seeds are kept dry and at low temperatures.

Storage temperatures between 35 and 50 °F (1.7 and 10 °C) are satisfactory when the moisture content of the seed is low. Storage conditions should be such that tomato seeds are kept dry. These guidelines apply to storage of packaged tomato seed at the laboratory as well as at the wholesaler's location.

If carefully packed and stored as recommended in these guidelines, tomato seedlings should remain viable for up to four years. However, to maximize the likelihood that tomato seedlings are viable when testing compost as per the methodology in this document, small orders should be made frequently rather than large, bulk orders made infrequently. If a bulk order has to be made, limit it to the minimum quantity.

ANNEX A: protocol for the field bean germination and plant growth response test (recommended for testing composts destined for use as a growing media)

The methodology for this field bean bioassay is based on the seed germination and plant growth response test specified in BSI PAS 100 (the BSI PAS 100 test)[4]. The methodology specified for the BSI PAS 100 test, including the methodology for collecting compost samples, shall be followed unless otherwise specified below.

Methodology

a. Thoroughly clean the area before each test is started.

b. For each test a minimum of 3.5 litres of peat-based growing medium (PBGM) and 1 litre of test mix sample (TS) are required, and must be prepared as described in the BSI PAS 100 test, with the following exception: once prepared, PBGM and TS shall be mixed in the ratio 2PBGM : 1TS to provide the test mix.

c. A control of PBGM is required for this test. A maximum of six test samples can be tested for every one PBGM control.

d. Fill lightly a 2 litre plastic pot to the top of the pot with the PBGM or test mix as appropriate, and firm with a spare pot.

e. Distribute evenly 10 field bean seeds (*Vicia faba* cv *Fuego*) on the surface and cover with spare PBGM or test mix as appropriate.

f. Place the pot onto a plastic saucer to ensure that any run off is collected and does not contaminate the area where the test is being conducted.

g. Watering can be applied overhead but splash must be avoided.

Note: since this test relies on scoring of individual plants, the use of replicate pots is not considered necessary. Users of the test may specify replication if they require it.

Assessments

a. Record the number of seeds germinated at 14 days. If:
   - fewer than 70% of seeds have germinated in the PBGM control, the test is invalid; and
   - if the test is valid, but fewer than 70% of seeds have germinated in the test mix, the sample will have failed[5].

b. Record the number of plants at 28 days after sowing.

c. Record the number of plants with visible symptoms of herbicide damage at 28 days after sowing. Visible symptoms should be categorised in accordance with the following:

   0 (nil) – no visible symptoms, plant growth and leaf development look normal compared to the PBGM control
   1 (Slight) - leaf curling on some newly developing or older leaves
   2 (Slight – moderate) – leaf curling and cupping on most leaves
   3 (Moderate) – leaf cupping on most leaves and slight twisting/distortion in the plant head
   4 (Moderate - severe) – leaf cupping of most leaves and moderate twisting of the plant head
   5 (Severe) – severe leaf cupping, and clearly abnormal growth of the plant head

   The photographs provided below should be used to decide the damage category.

d. Report on a per plant basis the number of plants in each applicable category of symptom severity at 28 days after sowing:

	0 (Nil)	**1 (Slight)**	**2 (Slight - moderate)**	**3 (Moderate)**	**4 (Moderate - severe)**	**5 (Severe)**
Number of plants						

[4] *At the time of publication, BSI PAS 100:2011 was the current version of the specification for composted materials.*

[5] *The germination requirements are different to the standard BSI PAS 100 plant growth response test because field bean is a slightly less reliable germinator than tomato.*

Test validity

The test is invalid if symptoms are observed in the PBGM grown plants.

As noted above, the test is invalid if after 14 days fewer than 70% of seeds germinate in the PBGM control.

Pass / failure

A sample will have passed the field bean germination and plant growth response test if all plants grown in the test mix exhibit symptoms of 2 or less.

A sample will have failed the field bean germination and plant growth response test if any plants grown in the test mix exhibit symptoms of greater than 2

Photographs illustrating characteristic symptoms on the 0-5 scale


2


3


4


5

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