SLOVENSKI STANDARD
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Rastlinski biostimulanti - Določanje mikoriznih gliv

Plant biostimulants - Determination of mycorrhizal fungi

Pflanzen-Biostimulanzien - Bestimmung von Mykorrhizapilzen

Biostimulants des végétaux - Détermination des champignons mycorhiziens

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65.080	Gnojila	Fertilizers

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Plant biostimulants - Determination of mycorrhizal fungi

Biostimulants des végétaux - Détermination des champignons mycorhiziens

Pflanzen-Biostimulanzien - Bestimmung von Mykorrhizapilzen

This European Standard was approved by CEN on 26 August 2024.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Contents

Page

European foreword

This document (EN 17722:2024) has been prepared by Technical Committee CEN/TC 455 “Plant biostimulants”, the secretariat of which is held by AFNOR.

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of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.

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

This document supersedes CEN/TS 17722:2022.

EN 17722:2024 includes the following significant technical changes with respect to CEN/TS 17722:2022:

— the European foreword has been updated;

— the Introduction has been updated;

— the Bibliography has been re-numbered;

— Clause 4 has been reworked;

— Clause 5 has been reworked;

— Clause 6 has been reworked;

— Annex A on repeatability and reproducibility of the method has been added;

— Annex ZA has been added.

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

For the relationship with EU Legislation, see informative Annex ZA, which is an integral part of this document.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations 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, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Introduction

The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European Standards or European Standardization deliverables to support the implementation of Regulation (EU) 2019/1009 of 5 June 2019 [1] laying down rules on the making available on the market of EU fertilising products ("FPR" or "Fertilising Products Regulation").

This standardization request, presented as SR M/564 and relevant amendments, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe". The interest in plant biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

1 Scope

This document specifies a horizontal method for the enumeration and genus/species determination [2], [3], [4] of mycorrhizal fungi in microbial plant biostimulants.

This document is applicable to the blends of fertilizing products where a blend is a mix of at least two of the following component EU fertilising products categories: Fertilizers, Liming Materials, Soil Improvers, Growing Media, Plant Biostimulants and where the following category Plant Biostimulants is the highest percentage in the blend by mass or volume, or in the case of liquid form by dry mass. If Plant Biostimulants is not the highest percentage in the blend, the European Standard for the highest percentage of the blend applies. In case a blend of fertilizing products is composed of components in equal quantity or in case the component EU fertilising products used for the blend have identical formulations[1], the user decides which standard to apply.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
arbuscular mycorrhizal fungus
AMF
AM fungus
biotrophic microscopic fungus belonging to the Glomeromycota [5] phylum (synonymous Glomeromycota) that establishes obligate symbiotic associations with more than 70 % of plant species on Earth

Note 1 to entry: Arbuscular mycorrhizal fungi produce structures inside plant roots, such as vesicles and/or endospores, but also specialized nutrient exchange structures called arbuscules.

Note 2 to entry: The hyphae do not penetrate the plant cell protoplast, but instead, they invaginate the cortical cell membrane where they branch dichotomously to develop the arbuscule. This is meant to be the place where the exchange of nutrients and water takes place between the plant and the fungus.

Note 3 to entry: The extraradical mycelium from arbuscular mycorrhizal fungi forms an extensive network within the soil, which increases plant nutrient availability and uptake.

[1] An example of such a blend is a product with 2 claimed functions consisting of a non-microbial plant biostimulant and an organic fertiliser composed of 1kg/kg of plant biostimulant from seaweed.

3.2
ectomycorrhiza
hyphal sheath, or mantle, covering the root tip and an extracellular Hartig net of hyphae surrounding the plant cells within the root cortex

Note 1 to entry: Beneficial symbiotic associations established by filamentous fungi belong mainly to the Ascomycota and Basidiomycota phyla with around 5 % to 10 % of coniferous and deciduous trees.

Note 2 to entry: In some cases, the hyphae can also penetrate into the plant cells, in which case the mycorrhiza is called an ectendomycorrhiza. Outside the root, the ectomycorrhizal extraradical mycelium forms an extensive network within the soil, which increases plant nutrient availability and uptake. Since these fungi have septate hyphae, hyphal fragments along with spores are considered long-term effective propagation structures.

3.3
endomycorrhiza
symbiotic association characterized by a filamentous fungal partner that colonizes the plants' root tissues intracellularly

EXAMPLE Four main groups of endomycorrhizal associations exist: arbuscular, ericoid, orchidoid and sebacinoid mycorrhizae.

3.4
ericoid mycorrhizal fungus
filamentous fungus belonging to the Ascomycota phylum that establishes endomycorrhizal symbiotic associations specifically with plants within the family *Ericaceae* (such as blueberry and cranberry)

Note 1 to entry: The intraradical growth phase is characterized by a dense coil of hyphae in the outermost layer of root cells. Ericoid mycorrhizal fungi also have saprotrophic capabilities, which can enable the plant to access nutrients in organic matter that would not yet be available to the plant.

3.5
in vivo
production performed in open area (greenhouse, tunnel, open field)

3.6
in vitro
production performed in monoxenic conditions

3.7
mycorrhiza
symbiotic relationship between a filamentous fungus and a plant

Note 1 to entry: In a mycorrhizal association, the fungus colonizes the plants' root tissues either intracellularly (as with endomycorrhiza) or extracellularly (as with ectomycorrhiza). This beneficial interaction brings several advantages to the plants such as, for instance, enhanced uptake of nutrients and water.

3.8
orchidoid mycorrhizal fungus
filamentous fungus belonging to the Basidiomycota phylum that establishes endomycorrhizal symbiotic associations specifically with plants within the family *Orchidaceae*

Note 1 to entry: The hyphae of orchidoid mycorrhizal fungi penetrate the root cell and form a dense coil of hyphae, where numerous exchanges take place.

3.9
propagule
component of the fungus able to initiate a symbiosis with plant roots

3.10
sebacinoid mycorrhizal fungus
endophytic filamentous fungus belonging to the Basidiomycota phylum, more specifically the order Sebacinales, which establishes mutualistic symbiotic relationship with a wide variety of plant hosts

EXAMPLE The model species *Piriformospora* spp.

Note 1 to entry: Sebacinoid mycorrhizal fungi colonize plant roots with an intracellular mycelium where nutrient exchanges take place.

3.11
serendipita mycorrhizal fungus
Serendipitaceae (formerly Sebacinales Group B) that belongs to a taxonomically, ecologically and physiologically diverse group in the phylum Basidiomycota (kingdom Fungus)

Note 1 to entry: While historically recognized as orchid mycorrhizae, recent phylogenetically-based studies have demonstrated both their widespread distribution and the broad spectrum of mycorrhizae form.

Note 2 to entry: Serendipita mycorrhizal fungi are associated to all families of herbaceous angiosperms (flowering plants) from temperate, subtropical and tropical regions.

Note 3 to entry: Serendipita mycorrhizal fungi should be considered as a previously hidden, but amenable and effective microbial tool for enhancing plant productivity and stress tolerance.

3.12
spore
unicellular dissemination cell formed by fungi in mycorrhiza

Note 1 to entry: Their size ranges from 50 µm to 500 µm.

3.13
Unit Potential Mycorrhizal
UPM
unit of counting for mycorrhizae

where

U is unit, spore or propagule able to initiate mycorrhizae formation in a host plant's root;

P is potential, since the development of the symbiosis depends on different factors (soil, plant, agriculture practises, competition with other soil borne microorganisms, etc.);

M is mycorrhizal, since the inoculum is able to initiate new mycorrhizae in association with plant roots, depending on the factors previously cited.

EXAMPLE UPM per gram (% spores, % propagules) (*in vivo*, *in vitro*).

4 Methods for the quantification of mycorrhizae

4.1 General

According to the type of mycorrhizae analysed (see Figure 1), the method to be used is listed in Table 1 to obtain the quantification in UPM.

The methods are:

Method N°1: Spore isolation and counting with MTT;

Method N°2 Procedure for the clearing and staining of the root specimens and the enumeration of spores/vesicles in the stained root samples. Only if the root fragments extraction is feasible from the formulation matrixes. If not possible, method N°3 shall be used;

Method N°3: Endomycorrhiza bioassay, the MPN test (Most Probable Number of mycorrhizal propagule);

Method N°4: Ectomycorrhizae and ericoid mycorrhizae count on plates.

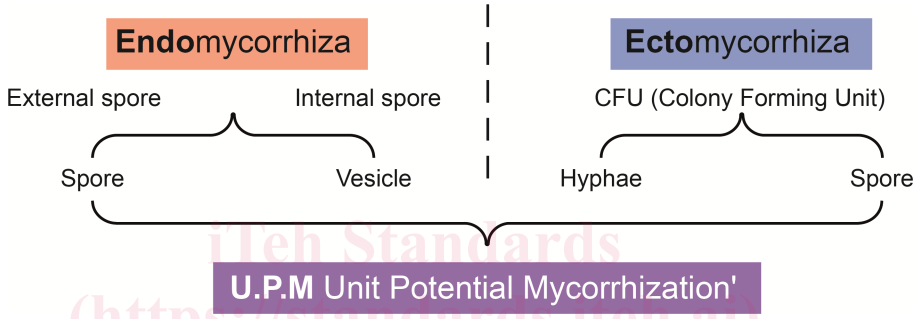

Figure 1 — Different types of mycorrhizas and propagules

Table 1 — Methods to use for the enumeration of UPM with plant cultures and without plant cultures

Origin of product	SPORES Extractable	Other propagules, roots extractable	Endo mycorrhizae	Ectomycorrhizae	Ericoid mycorrhizae	Orchidoid mycorrhizae	Sebacinoid mycorrhizae	Serendipita mycorrhizae
in vitro	Yes	NO	Method N°1	Method N°4	Method N°4			
in vitro	Yes	Yes	Method N°1 to count spores and Method N°2 to count propagules					
in vivo	NO	NO	Method N°3					
in vivo	Yes	NO	Method N°1	Method N°4	Method N°3			
in vivo	Yes	Yes	Method N°1 to count spores and Method N°2 to count propagules					
in vivo	NO	Yes	Method N°2			Method N°3	Method N°3	Method N°3

Note that the operator or laboratory needs to check with the manufacturer or distributor the nature of the sample (spore, root or mixture of both) in order to determine the most appropriate method.

4.2 How to prepare the initial sample

4.2.1 General

For solid U.P.M/ g and for liquid U.P.M / ml.

A base concentration for a product is 500 UPM/g. The whole preparation should be made according to this.

H High = higher than 100 000 UPM/g;

M Medium = between 1 000 and 100 000 UPM/g;

L Low = below 1 000 UPM/g.

For samples with different concentrations, different amounts of sample should be taken in a proportionate amount of tap water in order to maintain the proportion 1:10 as follows:

— for H, take 2,5 g in 22,5 ml of tap water;

— for M, take 25 g in 225 ml of tap water;

— for L, take 250 g in 2 250 ml of tap water.

A representative sample of the product shall be prepared according to the following procedure which takes into consideration the different formulations of plant biostimulants.

4.2.2 Liquid (water-based) formulations

Dispense the quantity of sample, depending on the concentration of the product as described in 4.2.1, into tap water maintained at room temperature in a flask and shake for 10 min or more until the distribution is optimal, with a magnetic stirrer at half speed.

4.2.3 Liquid (oil-based) emulsifiable concentrate (EC) formulations

Dispense the quantity of sample, depending on the concentration of the product as described in 4.2.1, into tap water maintained at room temperature in a flask and shake for 10 min or more until the distribution is optimal, with a magnetic stirrer at half speed.

4.2.4 Solid wettable powder (WP) formulations

Dispense the quantity of sample, depending on the concentration of the product as described in 4.2.1, into tap water maintained at room temperature in a flask and shake for 20 min or more until the distribution is optimal, with a magnetic stirrer at half speed.

4.2.5 Solid water dispersible granules (WDG) formulations

Dispense the quantity of sample, depending on the concentration of the product as described in 4.2.1, into tap water maintained at room temperature in a flask and shake for 40 min or more until the distribution is optimal, with a magnetic stirrer at half speed. If required, help the dispersion of the formulations with another apparatus such as a laboratory homogenizer[2] after having sieved (100 mesh sieve) the particles, and resuspend them in the same suspension [6].

[2] Stomacher® and Tween® 20 are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of these products.