# SLOVENSKI STANDARD
# SIST-TS CEN/TS 17722:2023

**01-februar-2023**

**Rastlinski biostimulanti - Določanje mikoriznih gliv**

Plant biostimulants - Determination of Mycorrhizal fungi

Pflanzen-Biostimulanzien - Bestimmung von Mykorrhizapilzen



Biostimulants des végétaux - Détermination des champignons mycorhiziens

**Ta slovenski standard je istoveten z: CEN/TS 17722:2022**



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TECHNICAL SPECIFICATION

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# Plant biostimulants - Determination of mycorrhizal fungi

Biostimulants des végétaux - Détermination des champignons mycorhiziens

Biostimulanzien für die pflanzliche Anwendung - Bestimmung von Mykorrhizapilzen

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The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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# European foreword

This document (CEN/TS 17722:2022) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

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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.

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# Introduction

This document was prepared by the experts of CEN/TC 455 "Plant Biostimulants". 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 the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products ("FPR" or "Fertilising Products Regulation"). This standardization request, presented as M/564, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe". Working Group 5 "Labelling and denominations" was created to develop a work program as part of this standardization request.

Technical Committee CEN/TC 455 "Plant Biostimulants" was established to carry out the work program that will prepare a series of standards. The interest in 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.

The biostimulants used in agriculture can be applied in multiple ways: on soil, on plants, as seed treatment, etc. A microbial plant biostimulant consists of a microorganism or a consortium of microorganisms, as referred to in Component Material Category 7 of Annex II of the EU Fertilising Products Regulation.

This document is applicable to all biostimulants in agriculture based on live microorganisms belonging to the mycorrhiza.



Table 1 summarizes many of the agro-ecological principles and the role played by biostimulants.

**Table 1 — Agro-ecological principles and the role played by biostimulants**



| |
|---|
| **Increase biodiversity** |
| By improving soil microorganism quality/quantity |
| **Reinforce biological regulation and interactions** |
| By reinforcing plant-microorganism interactions |
| — symbiotic exchanges i.e. *Mycorrhiza* |
| — symbiotic exchanges i.e. *Rhizobiaceae/Fava* |
| — secretions mimicking plant hormones (i.e. *Trichoderma*) |
| By regulating plant physiological processes |
| — e.g. growth, metabolism, plant development |
| **Improve biogeochemical cycles** |
| — improve absorption of nutritional elements |
| — improve bioavailability of nutritional elements in the soil |
| — stimulate degradation of organic matter |

**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 was developed to provide a horizontal method for enumeration and genera/species determination [1], [2], [3] of mycorrhizal fungi in plant biostimulants products in accordance with the EU Fertilising Products Regulation.

# 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 terminological databases for use in standardization at the following addresses:

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

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

**3.1**
**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, enhancement of nutrients and water uptake.



[SOURCE: CEN/TS 17724:2022, 3.2.2.6]



**3.2**
**endomycorrhiza**
symbiotic association characterized by a filamentous fungal partner that colonizes the plants' root tissues intracellularly

EXAMPLE Four main groups of endomycorrhizal associations exist like arbuscular, ericoid, orchidoid and sebacinoid mycorrhiza.

[SOURCE: CEN/TS 17724:2022, 3.2.2.6.1]

**3.3**
**arbuscular mycorrhizal fungus**
**AMF**
**AM fungus**
biotrophic microscopic fungus belonging to the Glomeromycota 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, which 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: Arbuscular mycorrhizal fungi extraradical mycelium forms an extensive network within the soil, which increases plant nutrient availability and absorption.

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

Note 1 to entry: The intraradical growth phase is characterized by 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 not yet available.

**3.5**
**orchidoid mycorrhizal fungus**
filamentous fungus belonging to the Basidiomycota phylum that establishes endomycorrhizal symbiotic associations specifically with orchids

Note 1 to entry: The hyphae of ochidoid mycorrhizal fungi penetrate the root cell and form dense coil of hyphae, where the nutrient exchange takes place.

**3.6**
**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 intracellular mycelium, where the nutrient exchanges take place.

**3.7**
**serendipita mycorrhizal fungus**
serendipitaceae (formerly Sebacinales Group B) belonging to a taxonomically, ecologically and physiologically diverse group of fungi in the Basidiomycota (kingdom Fungi)



Note 1 to entry: While historically recognized as orchid mycorrhizae, recent based phylogenetic studies have demonstrated both their pandemic distribution and the broad spectrum of mycorrhizal types they 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: Serendipitaceae mycorrhizal fungi should be considered as a previously hidden but amenable and effective microbial tool for enhancing plant productivity and stress tolerance.

**3.8**
**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 phylum with around 5 % to 10 % of coniferous and deciduous trees.

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

[SOURCE: CEN/TS 17724:2022, 3.2.2.6.2]

**3.9**
**spore**
very small and very tough cell able of germination under favourable conditions, caused by the fungi which ensure its dissemination

Note 1 to entry: There are sexual, asexual or vegetative spores [1].

**3.10**
**propagule**
component of the fungus able to initiate a symbiosis with root

**3.11**
***in vivo***
production performed in open area (greenhouse, tunnel, open field)

**3.12**
***in vitro***
production performed in monoxenic conditions

**3.13**
**Unit Potential Mycorrhizal**
**UPM**
unit of counting for mycorrhiza



where

**U** is unit, spore or propagule of any type able to initiate mycorrhiza formation in a host plant’s root;

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

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

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

## 4 Methods for the quantification of mycorrhiza

### 4.1 General

According to the type of mycorrhiza analysed (see Figure 1), the method to be used is listed in the Table 2 to obtain the quantification in UPM.

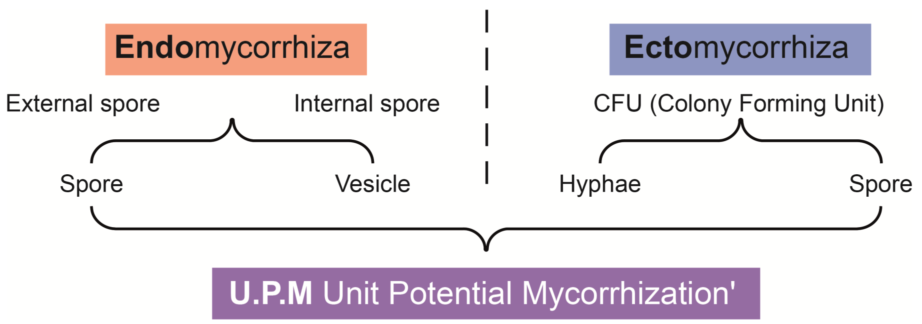


**Figure 1 — Different types of mycorrhizas and propagules**

**Table 2 — Methods to use for enumeration of UPM with plant cultures and without plant cultures**



| Origin of product | SPORES Extractable | Other propagules, roots extractable | Endo mycorrhiza | Ectomycorrhiza | Ericoid | Orchidoid | Sebacinoid | Serendipita |
|---|---|---|---|---|---|---|---|---|
| *in vitro* 1 | Yes | NO | Method N°1 | Method N°4 | Method N°4 | | | |
| *in vitro* 2 | Yes | Yes | Method N°1 to count the spores and Method N°2 to count propagules | | | | | |
| *in vivo* 1 | NO | NO | Method N°3 | | | | | |
| *in vivo* 2 | Yes | NO | Method N°1 | Method N°4 | Method N°3 | | | |
| *in vivo* 3 | Yes | Yes | Method N°1 to count the spores and Method N°2 to count propagules | | | | | |
| *in vivo* 4 | NO | Yes | Method N°2 | | | Method N°3 | Method N°3 | Method N°3 |

## 4.2 How to prepare the initial sample

### 4.2.1 General

A base concentration of a product is a product of 500 UPM/g. All the preparation should be made according to this.

H High concentration means higher than 100 000 UPM/g;

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

L Low means below 1 000 UPM/g.

For samples with different concentrations, different amounts 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 millilitre 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 biostimulants based products.

### 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 of 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 of 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 of 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 of 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 other apparatus such as a Stomacher®[1] after having sieved (100 mesh sieve) the particles and resuspend them in the same suspension.

[1] Stomacher® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.