International Rules for Seed Testing
Annexe to Chapter 7: Seed Health Testing Methods

7-007: Detection of *Botrytis cinerea* on *Linum usitatissimum* (Flax)

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DISCLAIMER: whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description ISTA shall not be liable for any loss damage etc., resulting from the use of this method.

Crop: *Linum usitatissimum* (Linseed, Flax)
Pathogen: *Botrytis cinerea* Pers. ex Pers. Perfect state: *Sclerotinia fuckeliana* (de Bary) Fuckel

Prepared by: ISTA-PDC Method Validation Sub-committee

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Submitted by: ISTA-PDC Method Validation Sub-committee

Background

This method was originally published in the ISTA Handbook of Seed Health Testing in November 1964 as S.3. No. 10, and revised in 1981 by C. Anselme and R. Champion, La Minière, France. This method appears in Annex 7.4.3.A.5 of the ISTA Rules 1999. It has been incorporated into the new Annexe to Chapter 7, Seed Health Testing Methods as method 7-007 and is subject to review before 2006.

Summary of Validation Study

Studied in International Comparative testing 1959. In a comparative study of the agar method described and a blotter method the test results obtained by the agar method were significantly higher than those obtained by the blotter method, particularly when infections were below 10 per cent (Anselme, Jailloux, and Champion, 1965).

Safety Precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation of media, autoclaving and weighing out of ingredients. It is assumed that this procedure is being carried out in microbiological laboratory by persons familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic technique. Dispose of all waste materials in an appropriate way (e.g. autoclave, disinfect) and in accordance with local safety regulations.

Treated Seed

This method may be influenced by treatment applied to the seed lot. Seed health tests on chemically treated seeds will generally deliver unreliable test results caused by masking or inhibition of the growth of the target organism.

Materials

Reference Material	-	The use of reference cultures or other appropriate material is recommended when ever possible.
Media	-	Malt Agar
Petri dishes	-	When sowing density is given by a number of seeds per petri dish, a diameter of 90 mm is assumed.
Incubator	-	Capable of operating in the range 20ºC ± 2ºC.

Sample Preparation

The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules.

Method

1. *Pretreatment*
   None
2. *Agar Plate Method*
   Malt agar containing 2% agar and 1% malt extract.
3. *Sowing density*
   Place 10 seeds on the agar surface in each petri dish.
4. *Incubation*
   7 days at 20ºC in darkness
5. *Examination*
   After 5 and 7 days examine for roots showing a soft rot and covered by abundant grey mycelium. Colonies on agar measure up to 5 cm in diameter after 5 days (Fig. 2). Identification can be checked by high power microscope (magnification x200). Mycelium of tape-like hyphae producing bunches of branching conidiophores with ovoid-hyaline one-celled conidia 8 x 11 - 6 x 19µ (Fig. 3). When analysts are familiar with the fungus, naked eye examination is sufficient for identification (Muskett and Malone, 1941; Tempe, 1963; Malone, and Muskett, 1964; Ellis and Waller, 1974).

General Methods (common to many test procedures)

1. Checking tolerances

   Tolerances provide a means of assessing whether or not the variation in result within or between tests is sufficiently wide as to raise doubts about the accuracy of the results. A tolerance table, which can be applied to agar plate tests, can be found in Table 5.1 of Annex 15 of the ISTA Rules or in Table G1 of the Handbook of Tolerances and Measures of Precision for Seed Testing by S. R. Miles (Proceedings of the International Seed Testing Association 28 (1963) No 3, pp 644).

2. Reporting Results

   The result of a seed health test should indicate the scientific name of the pathogen detected and the percentage of infected seeds. When reported on an ISTA Certificate results are entered under Other Determinations. The results should be accompanied by information on the test method used, including any pretreatment.

Preparation of Media

Malt Extract Agar

Compound	g/l	g/500 ml
Malt Extract	10	5
Agar (equivalent to oxoid No.3)	20	10
Deionized/Distilled Water	1l	500 ml

CCP *If using a commercial preparation ensure that it contains 2% agar and 1% malt extract.*

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 (or 500) ml of deionized/distilled water.
3. Dissolve powdered Malt Agar in deionized/distilled water by stirring.
4. Autoclave at 15 PSI and 121°C for 15 min.
5. Allow agar to cool to approx. 50°C.
6. Pour 15-22 ml of molten agar into 90 mm Petri plates and allow to solidify at room temperature before use.

Plates may be stored at room temperature in a dry cabinet or at 4ºC.

Quality Assurance

Critical Control Points

Where the wording of the original Working Sheet suggests that an action is critical this has been marked with ***CCP***.

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 10, C. Anselme and R. Champion, 1981.

Anselme, C., Jailloux, F. and Champion, R. (1965). Methode d'analyse sanitaire des semences de Lin. Proceedings of the International Seed Testing Association. 31, 157-167.

Champion, R. (1969). Quelques parasites importants transmis par les semences. Identification au laboratoire. Agriculture, 322, 3-8.

Ellis, M.B. and Waller, J.M. (1974). C.M.I. Descriptions of pathogenic fungi and bacteria No. 431. Commonwealth Mycological Institute, Kew.

Malone, J. P. and Muskett, A.E. (1964). Seed-borne fungi. Description of 77 fungus species. Proceedings of the International Seed Testing Association. 29, 177-384.

Muskett, A.E. and Malone, J.P. (1941). The Ulster method for the examination of flax seed for the presence of seed borne parasites. Annals of Applied Biology. 28, 8-13.

Tempe, J. de (1963). Health testing of flax seed. Proceedings of the International Seed Testing Association. 28, 107-131.

Fig. 1. Left: heavily diseased seed with dull grey aspect
Right: healthy seed with bright appearance

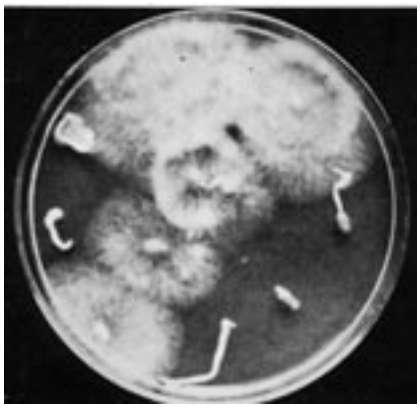

Fig. 2. Colonies of *Botrytis cinerea* spreading from diseased flax seed on malt agar after 5 days of incubation

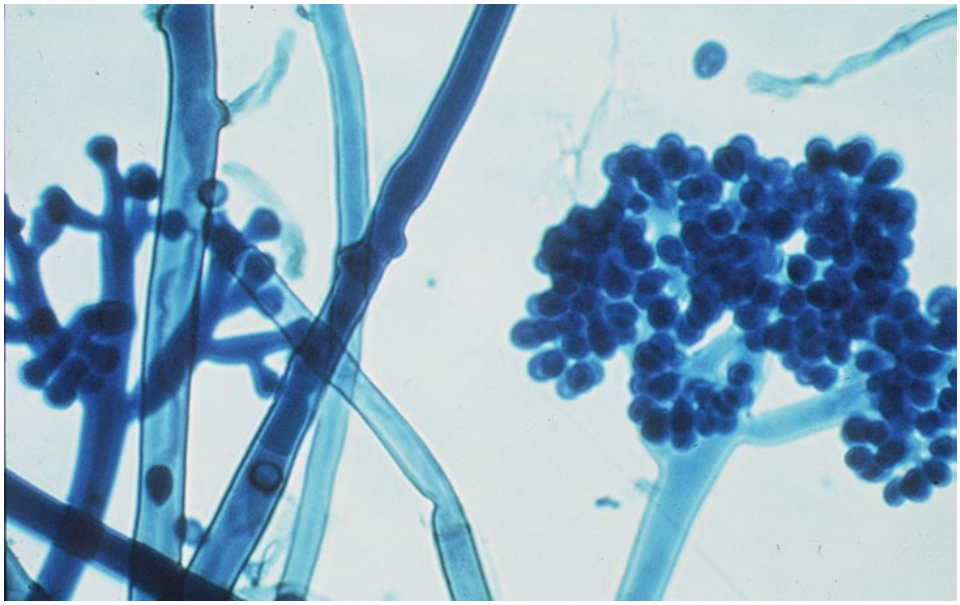

Fig. 3. Conidiophores and conidia of *Botrytis cinerea* and tapelike mycelium. x150