International Rules for Seed Testing 2024

Validated Seed Health Testing Methods

7-005: Detection of *Ascochyta pisi* in *Pisum sativum* (pea) seed

Including changes and editorial corrections adopted at the Ordinary General Meeting 2023 in Verona, Italy

Effective from 1 January 2024

Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at ista.office@ista.ch.

Please send comments, suggestions or reports of problems relating to this method to the ISTA Seed Health Committee, c/o ISTA Secretariat.

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 or damage, etc. resulting from the use of this method.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during weighing out of ingredients. It is assumed that persons carrying out this test are in a laboratory suitable for carrying out microbiological procedures and familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic techniques. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French, German and Spanish versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.

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7-005: Detection of *Ascochyta pisi* in *Pisum sativum* (pea) seed

Host: *Pisum sativum* L.s.l.
Pathogen(s): *Ascochyta pisi* Lib.

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Revision history

Version 1.0, 2000-07-13
Revised 2001-19-11: J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.1, 2008-01-01: Treated seed revised; Reporting results revised
Version 1.2, 2017-01-01: Reporting results revised
Version 1.3, 2021-01-01: Sample preparation changed to Sample size and paragraph revised; Media and solutions revised
Version 1.4, 2022-01-01: Methods (pretreatment) revised
Version 1.5, 2024-01-01: Sample size and Figures revised

Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 16 revised 1987 by P.D. Hewett, Official Seed Testing Station for England and Wales, Cambridge, United Kingdom. The method was incorporated into the newly revised *Annexe to Chapter 7* in 2002 from the 1999 edition of the ISTA Rules. The method was reviewed by the ISTA-Seed Health Committee in 2006 (Cockerell & Koenraadt, 2007) with the recommendation to accept for a further five years.

Treated seed

This method has not been validated for the determination of *Ascochyta pisi* on treated seed. Seed treatments may affect the performance of the method.

(Definition of treatment: any process, physical, biological or chemical, to which a seed lot is subjected, including seed coatings. See 7.2.3)

Sample size

The sample size (total number of seeds to be tested) depends on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The minimum sample size should be 400 seeds.

Materials

Reference material: reference cultures or other appropriate material
Media: malt agar or potato dextrose agar
Sodium hypochlorite solution (1 % available chorine): for seed disinfection
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 20 ±2 °C.

Methods

1. Pretreatment: Immerse seeds in a solution of sodium hypochlorite (NaOCl) (1 % available chlorine) for 10 min, then drain, rinse well in sterile water and drain again.
2. Agar method: Malt or potato dextrose agar. Place 10 seeds on the agar surface in each Petri dish.
3. Incubation: 7 days at 20 °C in darkness.
4. Examination: After 7 days examine each seed by naked eye for abundant white mycelium which often covers infected seeds (Fig. 1). Doubtful colonies may be confirmed by the presence of wavy hyphae at the edge of the colony when examined at ×25 magnification. Colony diameter typically 20–30 mm, occasionally smaller or incompletely surrounding the seed. Reverse of colonies medium to dark orange-brown centrally, opaque and even, becoming lighter in colour towards the edge of the colony. Gelatinous-looking orange-brown pycnidia often present (although only sometimes clearly visible), particularly where seed touches agar. Under STM at ×20–25 magnification, using both transmitted and incident light, hyphae are curled, often several running together, typically with moisture drops (although these evaporate easily) (Fig. 2). Very limited growths from some seeds may only be seen if dishes tilted to get lighting at best angle, or under STM examination

or after extended incubation. Pycnidia are up to 250 µm in diameter. Spores, hyaline, cylindric, of slightly curved with rounded ends, 1-septate, slightly constricted at septum, mostly 12 × 4.5 µm (Punithalingam & Holliday, 1972).

Notes: Samples frequently bear *A. pinodes* (*Mycosphaerella pinodes* (Berk. & Blox.) Westerg.) and, occasionally, *A. pinodella* (*Phoma medicaginis* Malbr. & Roum. var. *pinodella* (Jones) Boerema). These pathogens differ markedly from *A. pisi* in their colony and mycelial characters and in spore morphology (see CMI descriptions Nos. 340 and 518, respectively).

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are sufficiently wide as to raise doubts about the accuracy of the results. Suitable tolerances, which can be applied to most direct seed health tests, can be found in Table 5B Part 1 of Chapter 5 of the ISTA Rules, or Table G1 in Miles (1963).

Reporting results: The result of a seed health test should indicate the scientific name of the pathogen detected and the test method used. When reported on an ISTA Certificate, results are entered under 'Other Determinations'.

The report must indicate the number of seeds tested.

In the case of a negative result (pathogen not detected), the results must be reported as 'not detected'.

In the case of a positive result, the report must indicate the percentage of infected seeds.

Quality assurance

Critical control points (CCP)

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

Media and solutions

Sodium hypochlorite solution

Sodium hypochlorite for pretreatment of seed can be prepared from commercial bleach diluted to 1 % available chlorine. The concentration of chlorine in commercial bleach varies considerably and declines with storage. Use the formula:

$$V_{stock} = V_{final} \times C_{final} / C_{stock}$$

(where V = volume and C = % available chlorine) to calculate the volume of commercial bleach stock solution required to prepare sodium hypochlorite solutions for use in seed pretreatment.

To prepare a 1 l solution of sodium hypochlorite containing 1 % chlorine from a stock of commercial bleach containing 12 % available chlorine:

$$V_{stock} = 1 \times 1/12 = 0.083$$

Thus add 83 ml of the 12 % stock to 917 ml water.

The percentage of active chlorine decreases rapidly in solution so, NaClO 1 % solution must be stored in the dark and used within 3 days of preparation. It is possible to check chlorine concentration with chlorine strip tests.

Malt agar

Malt agar[1]: according to manufacturer's instructions
Distilled/deionised water: 1000 ml

[1] Malt agar constituents should be equivalent to those of the manufacturers BD, USA or Oxoid, UK (CCP)

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Dissolve powdered malt agar in distilled/deionised 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 before use.

Storage

Prepared plates may be stored at 4 °C for up to 6 weeks.

Figure 1. Colonies of *Ascochyta pisi*, face (left) and reverse (right), from test on PDA, following hypochlorite pretreatment. Incubation for 7 days at 21 °C in darkness.

Figure 2. Colony of *Ascochyta pisi* on malt agar.

Figure 3. Conidia of *Ascochyta pisi*.

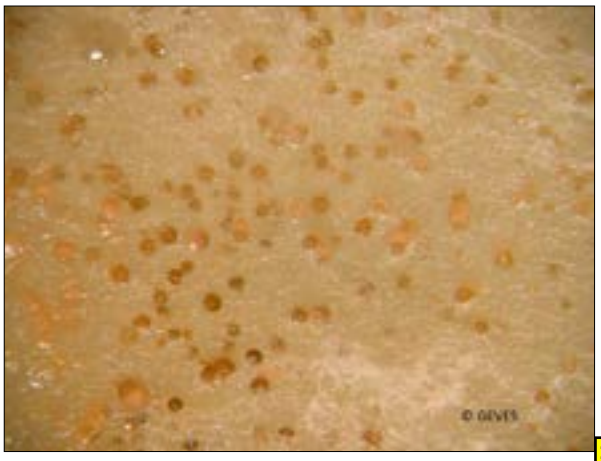

Figure 4. Pycnidia of *Ascochyta pisi*.

Potato dextrose agar

Potato dextrose agar[1]: according to manufacturer's instructions
Distilled/deionised water: 1000 ml

[1]Potato dextrose agar constituents should be equivalent to those of the following manufacturers BD, USA or Oxoid, UK (CCP)

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 (or 500) ml of distilled/deionised water.
3. Dissolve powdered PDA in distilled/deionised 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 before use.

Storage

Prepared plates may be stored at 4 °C for up to 6 weeks.

References

The following references are extracted from the *ISTA Handbook of Seed Health Testing*, Working Sheet No. 16, P. D. Hewett, 1987.

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Validation references

Studied in international comparative testing: 1960, 1966, 1967, 1968–71, 1973–1975

Agar tests detect approximately 50 % more infection by *Ascochyta* spp. than blotter tests (Anselme & Champion, 1962; Tempe, 1968).

International comparative tests (Hewett, 1987) showed that of over 350 results obtained by experienced stations, 95 % fell within tolerance limits used for germination tests.