

Antifungal Activity, Assessment on Textile Materials: Mildew and Rot Resistance of Textile Materials

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1. General Purpose and Scope

1.1 The two purposes of this test method are to determine the susceptibility of textile materials to mildew and rot and to evaluate the efficacy of fungicides on textile materials.

2. Principle

2.1 Tests I, II, III and IV can be used, singly or in combination, depending on the type of exposure to which the textile material will be subjected. For example, if the final product will come in contact with soil, Test I, which simulates this exposure, should be used; if the finished product will never come in contact with soil or tropical conditions, a much less severe test (e.g., II or III) should be used. Test II is specifically designed for cellulose-containing materials while Test III is for all others. For all materials intended for outdoor and above ground use, Test IV should be used. The two important considerations when evaluating textile materials in relation to fungal growth are (1) the actual deterioration of the textile product (rot), and (2) growth not necessarily deteriorating the product but making it unsightly (mildew) often with an unpleasant and musty odor.

2.2 Certain pre-exposures of textile products may be indicated when specific end-uses are critical (see Appendix A). When the end-use will be near high temperature and the fungicide may be volatile, a preliminary oven exposure may be desired. When the end-use will be in tropical exposures or outside with rainfall present, a leaching exposure should be performed before mildew evaluation is made. When at all possible, the textile material should be exposed to the expected conditions of use prior to performing this test.

3. Terminology

3.1 **mildew resistance**, n.—in *textiles*, resistance to development of unsightly fungal growths and accompanying unpleasant, musty odors on textile materials exposed to conditions favoring such

growths.

3.2 **rot resistance**, n.—in *textiles*, resistance to deterioration of a textile material as a result of fungal growth in or on it.

NOTE: Such deterioration is normally assessed by measuring loss in tensile strength.

4. Safety Precautions

NOTE: These safety precautions are for information purposes only. The precautions are ancillary to the testing procedures and are not intended to be all inclusive. It is the user's responsibility to use safe and proper techniques in handling materials in this test method. Manufacturers MUST be consulted for specific details such as material safety data sheets and other manufacturer's recommendations. All OSHA standards and rules must also be consulted and followed.

4.1 This test should be performed only by trained personnel. The U.S. Department of Health and Human Services publication *Biosafety in Microbiological and Biomedical Laboratories* should be consulted (see 24.1).

4.2 CAUTION: Some of the fungi used in these tests are allergenic and pathogenic; i.e., capable of infecting humans and producing disease. Therefore, every necessary and reasonable precaution must be taken to eliminate this risk to the laboratory personnel and to personnel in the associated environment. Wear protective clothing, respiratory protection, and impervious gloves when working with the organisms. NOTE: Choose respiratory protection that prevents penetration by the spores.

4.3 Good laboratory practices should be followed. Wear safety glasses in all laboratory areas.

4.4 All chemicals should be handled with care.

4.5 An eyewash/safety shower should be located nearby for emergency use.

4.6 Sterilize all contaminated samples and test materials prior to disposal.

4.7 Exposure to chemicals used in this procedure must be controlled at or below levels set by government authorities (e.g., Occupational Safety and Health Administration's [OSHA] permissible exposure limits [PEL] as found in 29 CFR 1910-1000 of January 1, 1989). In addition, the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) comprised of time weighted averages (TLV-TWA),

short term exposure limits (TLV-STEL) and ceiling limits (TLV-C) are recommended as a general guide for air contaminant exposure which should be met (see 24.2).

Test I Soil Burial

5. Scope

5.1 This procedure is generally considered to be the most severe test for textile products. Only those specimens that will come in direct contact with soil—such as sandbags, tarpaulins, tents—need to be tested by this procedure. It can also be used for testing experimental textile fungicides.

6. Test Specimens

6.1 Prepare the fabric specimens with dimensions $15.0 \pm 1.0 \times 4.0 \pm 0.5$ cm ($6.0 \pm 0.4 \times 1.5 \pm 0.2$ in.) with the long dimension parallel to the warp and unraveling to 2.5 ± 0.1 cm width (1.0 ± 0.04 in.), or, in the case of fabric with less than 20 threads per 2.5 cm (1.0 in.) to a predetermined number of threads to give a specimen 2.5 ± 1.0 cm in width (1.0 ± 0.4 in.). A sample cutter can also be used (see 24.3). The number of specimens will vary according to the number of variables. The suggested number of specimens is five for each treatment, control and reference fabric.

7. Test Procedure

7.1 Viability control: Expose untreated cotton cloth 271 g/m^2 (8 oz/yd²) in the soil bed for seven days during the test period to verify fungal activity. The soil bed shall be considered as satisfactory if the viability control fabric loses 90% breaking strength after seven days exposure.

7.2 Soil Bed: Place the air-dry test soil (see 24.4) in trays, boxes or suitable containers to a depth of 13.0 ± 1.0 cm (5.1 ± 0.4 in.) and bring to optimum moisture content by gradual addition of water accompanied by mixing to avoid puddling. After allowing it to stand for 24 h, sieve it through a 6.4 mm (0.25 in.) mesh screen. Maintain uniform moisture content by covering the soil container with a suitable lid. The moisture content of the soil during the test period shall be maintained between $25 \pm 5\%$ (based on dry weight). If the surrounding air is maintained at higher than $83 \pm 3\%$ relative humidity,

the loss of moisture is negligible.

7.3 Incubation: Bury the specimens horizontally on 10.0 ± 1.0 cm (3.9 ± 0.4 in.) of soil, spaced at least 2.5 cm (1.0 in.) apart and then cover with 2.5 ± 0.5 cm (1.0 ± 0.2 in.) of test soil. Incubation periods can vary from 2-16 weeks, depending on severity of service requirements, and other factors of importance to the interested parties. Maintain the temperature at $28 \pm 1^\circ\text{C}$ ($82 \pm 2^\circ\text{F}$) during the test period.

8. Evaluation and Report

8.1 Strength loss determination: Remove specimens, gently wash with water, dry at room temperature for 22 ± 4 h and then condition in an atmosphere of $64 \pm 2\%$ humidity and a temperature of $24 \pm 3^\circ\text{C}$ ($76 \pm 6^\circ\text{F}$) for 24 h. Determine breaking strength by the method outlined in ASTM D 5035, Standard Test Method for Breaking Force and Elongation of Textile Fabrics (Strip Force), using 25×75 mm (1×3 in.) jaw faces. The gage length is determined as 25% of the specimen length. Testing can be performed every two weeks or as specified by the end-user.

8.2 Report: Report the length of exposure to the soil bed, percent retained breaking strength when compared to the unexposed textile, any pre-exposure of specimens before burying and percent retained breaking strength of untreated specimen and/or viability control.

Test II

Agar Plate, *Chaetomium Globosum*

9. Scope

9.1 This procedure is used for evaluating rot resistance of cellulose-containing textile materials that will not come in contact with soil. It may also be used for determining uniformity of fungicide treatment.

10. Test Specimens

10.1 Proceed as in Section 6, if strength loss is to be determined. If only a visual examination is performed, a minimum of five samples is required. However, any number can be tested depending on end-users request. Cut 3.8 ± 0.5 cm (1.5 ± 0.2 in.) diameter discs from both treated and untreated samples.

11. Test Procedure

11.1 Organism: *Chaetomium globosum*. American Type Culture Collection No. 6205 (see 24.5).

11.2 Culture medium (see 24.6): The mineral salts agar should have the following composition:

Ammonium nitrate, NH_4NO_3 3.0 g
Potassium dihydrogen phosphate,
 KH_2PO_4 2.5 g

Dipotassium hydrogen phosphate,
 K_2HPO_4 2.0 g
Magnesium sulfate,
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g
Ferrous sulfate,
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g
Agar..... 20.0 g
Distilled water.....to 1000 mL

11.3 Inoculum: Place agar solution in any desired container such as test tube, French square bottle, Erlenmeyer flask, or petri dish. Sterilize in an autoclave at 103 kPa (15 psi) and 121°C (250°F) for 15 min and cool in a position which affords maximum inoculation surface. After the agar has hardened, under aseptic conditions, place on its surface a disc of filter paper previously sterilized by dry heat in an oven at $71 \pm 3^\circ\text{C}$ ($160 \pm 5^\circ\text{F}$) for 1 h. Streak the filter paper with spores of *Chaetomium globosum* by use of a sterile needle. Incubate at $28 \pm 1^\circ\text{C}$ ($82 \pm 2^\circ\text{F}$) for approximately 10-14 days to produce abundant growth. Remove the filter paper from the container and add it to 50 ± 2 mL of sterile distilled water containing a few glass beads and shake vigorously to bring the spores into suspension. Use this suspension for inoculum in 11.5.

11.4 Culture chamber: Melt mineral salts agar of the composition specified in 11.2 in an autoclave and distribute into any convenient container. Sterilize under conditions given in 11.3 and leave undisturbed until the agar hardens.

11.5 Inoculation: Pre-wet the specimens (but do not rub or squeeze) in water containing 0.05% of a nonionic wetting agent (see 24.7) and place in contact with the hardened agar medium in each container under aseptic conditions. Distribute 1.0 ± 0.1 mL of the inoculum evenly over the $15.0 \pm 1.0 \times 4.0 \pm 0.5$ cm ($6.0 \pm 0.4 \times 1.5 \pm 0.2$ in.) specimens by means of a sterile pipette. Use 0.2 ± 0.01 mL of inoculum for the 3.8 ± 0.5 cm (1.5 ± 0.2 in.) discs. Set up a control specimen, cellulose filter paper or untreated control, in a similar way by using 1.0 ± 0.1 or 0.2 ± 0.01 mL of sterile water.

12. Evaluation and Report

12.1 Strength loss evaluation: Proceed as per 8.1 and report the change in breaking strength as compared to the sample before exposure or the control if available.

12.2 Visual assessment: Report the extent of fungal growth on the discs, using a microscope (50X) where necessary, in accordance with the following scheme:

Observed Growth

No growth

Microscopic growth (visible only under the microscope)

Macroscopic growth (visible to the eye)

Test III Agar Plate, *Aspergillus Niger*

13. Score

13.1 Certain fungi, of which *Aspergillus niger* is one, can grow on textile products without causing measurable breaking strength loss within a laboratory experimental time frame. Nonetheless, their growth may produce undesirable and unsightly effects. This procedure is used to evaluate textile specimens where growth of these fungi is important.

14. Test Specimens

14.1 Cut duplicate 3.8 ± 0.5 cm (1.5 ± 0.2 in.) diameter discs from both treated and untreated samples. Other shapes and sizes can be used provided any anticipated size of the growth-free zone is taken into consideration.

15. Test Procedure

15.1 Organism: *Aspergillus niger*, American Type Culture Collection No. 6275 (see 24.5).

15.2 Culture medium: Proceed as per 11.2. Other suitable media are Czapek (Dox) Agar and Sabouraud Dextrose Agar (see 24.8).

15.3 Inoculum: Add scrapings from a ripe (7-14 days) fruiting culture of *Aspergillus niger* grown on the medium described in 11.2 containing $3.0 \pm 0.1\%$ glucose, to a sterile Erlenmeyer flask containing 50 ± 1 mL of sterile water and a few glass beads. Shake the flask thoroughly to bring the spores into suspension. Use this suspension as the inoculum.

15.4 Inoculation: If the test medium contains glucose, distribute evenly 1.0 ± 0.1 mL of the inoculum over the surface of the agar. Pre-wet the discs (but do not rub or squeeze) in water containing 0.05% of a nonionic wetting agent (see 24.7) and place on the agar surface. Distribute evenly over each disc 0.2 ± 0.01 mL of the inoculum by means of a sterile pipette. If the test medium does not contain glucose, a negative control fabric is required to ensure inoculum viability. Incubate all specimens at a temperature of $28 \pm 1^\circ\text{C}$ ($82 \pm 2^\circ\text{F}$) for 14 days when mineral salts agar is used and for seven days when 3% glucose is added to the mineral salts agar.

16. Evaluation and Report

16.1 At the end of the incubation period, report the percentage of surface area of the discs covered with the growth of *Aspergillus niger*, using a microscope (50X) where necessary, in accordance with the following scheme: