

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

Developed in 1946 by AATCC Committee RA31; revised 1952, 1957, 1971, 1981, 1987, 1988 (with title change), 1993, 1999, 2017; reaffirmed 1970, 1974, 1979, 1989, 1998, 2013; editorially revised and reaffirmed 1986, 2004; editorially revised 2010, 2019.

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, can 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) can be used. Test II is specifically designed for cellulose-containing materials while Test III is for all others. For materials intended for outdoor and above ground use, Test IV can 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 (mildewy), often with an unpleasant and musty odor.

2.2 Certain pre-exposures of textile products may be needed when specific end-uses are critical (see Appendix A). When the end-use will be in high temperature environments 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 **fungicide**, n.—a compound used to

kill fungi.

3.2 **fungistatic**, adj.—inhibiting the growth of fungi or fungal spores without destroying them.

3.3 **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.

3.4 **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 44.1).

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; see web site: www.osha.gov for latest version). 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 44.2).

Test I Soil Burial

5. Purpose and Scope

5.1 This procedure is used to evaluate the loss of strength of textile materials after exposure to soil and soil microorganisms. 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. Principle

6.1 Test specimens are buried in a container of soil. After a defined incubation period, the test specimens are removed and evaluated for loss of strength.

7. Referenced Documents

7.1 ASTM D5035, Standard Test Method for Breaking Force and Elongation of Textile Fabrics (Strip Method) (see 44.3).

8. Apparatus, Reagents, Materials

8.1 Untreated cotton cloth, 271 ± 20 g/m² (8 oz $\pm$ 0.6 oz/yd²). For viability control.

8.2 Soil. See 44.4 for acceptable soil types.

8.3 Incubator. Incubating equipment to maintain a temperature of $28 \pm 1^\circ\text{C}$ ($82 \pm 2^\circ\text{F}$).

8.4 Apparatus, reagents, and materials required to perform breaking strength by method ASTM D5035.

8.5 Autoclave. To sterilize test materials prior to disposal.

9. Specimens

9.1 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 wide (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 wide (1.0 ± 0.4 in.). A sample cutter can also be used. The number of specimens will vary according to the number of variables. The suggested number of replicate specimens is 5 (i.e., 5 specimens for each treatment, 5 controls and 5 reference fabrics).

10. Procedure

10.1 Soil Bed: Place the air-dry test soil (see 44.4) 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 at $25 \pm 5\%$ (based on dry weight). If the surrounding air is maintained at higher than $83 \pm 3\%$ relative humidity, the loss of moisture will be negligible.

10.2 Viability control: Expose untreated cotton cloth (see 8.1) in the soil bed for seven days during the test period to verify fungal activity. The soil bed shall be considered satisfactory if the viability control fabric loses 90% breaking strength after seven days exposure.

10.3 Incubation: After the soil bed has been determined to be satisfactory based on the viability control, place 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 to 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.

10.4 Do not reuse soil as components from the material(s) tested may be present in the soil bed and may impact future tests. Fresh soil must be used for each new test.

11. Interpretation, Calculation, and Evaluation

11.1 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 D5035, 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.

12. Report

12.1 The report shall include the following:

- 12.1.1 The test method and test part.
- 12.1.2 Composition of the soil bed.
- 12.1.3 Number of test specimen replicates used.
- 12.1.4 Incubation time period in the soil bed.
- 12.1.5 Percent retained breaking strength when compared to the unexposed textile.
- 12.1.6 Any pre-exposure of specimens before burying and percent retained breaking strength of untreated specimen and/or viability control.

13. Precision and Bias

13.1 The precision and bias of this test method have not been established. Refer to the precision and bias statement given in ASTM D5035.

Test II Agar Plate, *Chaetomium globosum*

14. Purpose and Scope

14.1 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.

15. Principle

15.1 Test specimens are placed on an agar surface and inoculated with fungi. After a defined incubation period, the test specimens are evaluated for loss of strength and/or observed for fungal growth.

16. Referenced Documents

16.1 ASTM D5035, Standard Test Method for Breaking Force and Elonga-

tion of Textile Fabrics (Strip Method) (see 44.3).

17. Uses and Limitations

17.1 If the test specimen is thick or non-porous, the fungal spores may not have access to minerals in the agar plate. This may cause a lack of fungal growth on the test specimen and cause misleading results.

18. Apparatus

18.1 Autoclave. To sterilize media and to sterilize test materials prior to disposal.

18.2 Incubator. Incubating equipment to maintain a temperature of $28 \pm 1^\circ\text{C}$ ($82 \pm 2^\circ\text{F}$).

18.3 Stereo microscope.

18.4 Counting chamber suitable for determining spore concentrations; e.g., hemocytometer.

18.5 Apparatus, reagents, and materials to perform breaking strength by method ASTM D5035 (optional).

19. Reagents and Materials

19.1 Organism: *Chaetomium globosum*. American Type Culture Collection No. 6205 (see 44.5).

19.2 Ammonium nitrate, NH_4NO_3 .

19.3 Dipotassium hydrogen phosphate, K_2HPO_4 .

19.4 Ferrous sulfate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

19.5 Magnesium sulfate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

19.6 Potassium dihydrogen phosphate, KH_2PO_4 .

19.7 Agar.

19.8 Cellulose filter paper.

19.9 Non-ionic wetting agent (see 44.6).

19.10 Gauze or glass wool, sterile.

19.11 Sterile scraping instrument to harvest fungal spores. Platinum or nichrome wire, plastic loop or needle, glass rod, or swab.

20. Specimens

20.1 If strength loss is to be determined, prepare specimens as in Section 9. If only a visual examination of fungal growth is required, cut 3.8 ± 0.5 cm (1.5 ± 0.2 in.) diameter discs from both treated and untreated samples. If the fabric is different on both sides, face up and face down, shall be tested. Five replicate specimens should be used; however, any number can be tested depending on end-users request.