DEVELOPMENT OF A STANDARD TEST METHOD FOR EVALUATION OF INSECTPROOFNESS OF WOOLLEN TEXTILES

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ABSTRACT

Factors governing the standardization of test methods for the assessment of insectproofness of woollen textiles have been critically examined. A standard test method using the woolly bear, *Anthrenus flavipes* Lec. and the case-bearing clothes moth, *Tinea pellionella* Linn. has been developed.

INTRODUCTION

Insect attack on woollen materials is caused by several species [1-3] of Dermestid beetles and moths and results in enormous economic loss[4]. Among beetles, the woolly bear-*Anthrenus flavipes* Lec. and the carpet beetle, *Attagenus piceus* Oliv. and among moths, *Tinea pellionella* Linn. and the webbing clothes moth, *Tineola bisselliella* Hum. are important. Of these, *A. flavipes* and *T. pellionella* are widely occurring species of wool destroying insects in India.[5,6]

The importance of protectants against the wool destroying insects and the need to assess the proofness are well recognized. However, at present no universal standard method exists and different methods are currently in use in different countries for the evaluation of insectproofness of woollen textiles. The object of this paper is to discuss the important factors which should be considered for the standardization and to present results of investigations carried out in this laboratory for the development of a standard insectproofness test method.

FACTORS GOVERNING STANDARDIZATION

Test insects

Although the larvæ of several species of moths and beetles attack woollen materials the only species which appear so far to have found favour as test insects in different parts of the world are *T. bisselliella*, *T. pellionella*, *A. piceus and A. flavipes*. The choice of larvæ of any of these species for the test method would appear to have been primarily dictated by the larger prevalence and incidence of attack by the species in the particular country in which the test method was developed. According to Meeuse[7] the test organism must be representative of all wool destroying insects, easily bred in the laboratory, should have short life-cycle and also should severely attack unproofed wool. In the U.K. [1,2,8] and Switzerland [9] current procedures make use of *T. bisselliella* only. In the U.S.A. [10] and Canada[11] in addition to *T. bisselliella* the black carpet beetle, *A. piceus* is also used. In Australia[12] and Newzealand,[13] *T. pellionella* has been found suitable.

Obviously it will be ideal to use, in any test procedure, larvæ of as many as possible of the species of beetles and moths that attack wool. Since in India damage to woollen stores in Service depots is mainly due to the attack by *A. flavipes* and *T. pellionella*, these species have been adopted in the insectproofness test method developed in this laboratory.

Age of larvæ

It is of importance that the larvæ used in the test should not vary in respect of their capacity to feed and that they also should not pupate during the course of the test. In the Swiss[9] method this has been achieved by using larvæ of *T. bisselliella* of size 3 to 5 mm and larvæ of *A. flavipes* of average weight of 0·8±0·1 mg. The American[10] practice is to use 25 to 27 days old larvæ of *T. bisselliella*. In the Canadian[11] and American[10] methods, in regard to *A. piceus*, larvæ weighing 4·5 to 6·5 mgm are used for the test. In U.K., Switzerland, Germany and Netherland [8], 21 to 28 days old larvæ of *T. bisselliella* are employed.

It was considered that consistent with the practice in standard bioassay adopted methods [14] for assessment of contact toxicity of insecticides, it will be far more satisfactory to employ larvæ of known age rather than particular range of weight or dimension. In order to select the most suitable age of larvæ for the insectproofness test method developed in this laboratory, investigations were carried out in respect of larvæ of *A. flavipes* and *T. pellionella*. In this, the relationship between larval age and extent of damage caused to woollen fabric, weight of excrement, mortality of larvæ and incidence of pupation during the course of the experiment were studied. The results are shown in Tables I and II. It will be observed that in case of *A. flavipes* the greatest damage is caused by age groups higher than 16 weeks, but since pupation during the test is to be avoided and since even the age group 14-16 weeks is not free from risk of pupation, the age group 12-14 weeks is most suitable for use in standard method. In regard to *T. pellionella* the most suitable age is 10 days, the reasons governing the selection being similar to those advanced for *A. flavipes*.

TABLE I

AGE OF THE LARVAE OF *A. flavipes* IN RELATION TO THE DAMAGE CAUSED TO FABRICS*

Age of Larvae (Weeks)	Number of Replicates	Loss in weight (Per cent)	Weight of Excrement (mgm)	Survival of Larvae (Per cent)	Visual Damage
16-20	15	24·0	59·8	98†	Severe
16-18	15	22·7	61·3	99	Severe
14-16	12	17·3	42·4	98†	Severe
12-14	12	21·0	51·3	99	Severe
10-12	6	14·5	39·6	99	Moderate
8-10	6	11·5	27·7	99	Moderate
6-8	3	12·0	23·6	100	Moderate
4-6	3	12·0	22·5	100	Moderate

* All-wool worsted white serge, 1·37 m-wide, weighing 388 gmδ per linear meter; Size of test strip, 5 σ. × 2·5 cmδ; Number of larvae exposed, 25; Period of exposure, 15 days; Temperature/Humidity, 27·8 ± 1·7°C/66 ± 12 per cent.

† One larva pupated in one replicate.

Experiments were also carried out to confirm that reproducible results are obtained on different occasions in respect of loss of weight of fabric and other characteristics where larvæ of the selected ages are used over a period of 15 days. The results are shown in

TABLE II

AGE OF THE LARVAE OF *T. Pellionella* IN RELATION TO THE DAMAGE CAUSED TO THE FABRIC

Age of Larvæ (Days)	Period of Exposure (Days)	Loss in weight (Per cent)	Weight of excrement (mgm)	Visual Damage
1	8	1·5	2·2	Slight
1	10	3·5	4·5	Slight
10	8	3·9	5·8	Slight
10	10	4·9	6·6	Slight
10	15	12·2	15·3	Moderate
15	10	13·7	28·2	Moderate
15	15†	22·5	36·0	Severe
20	8†	12·7	19·3	Moderate
20	10†	16·2	42·3	Severe
20	15†	27·0	42·0	Severe
25	8†	19·7	34·4	Severe

* All-wool worsted white serge, 1·37, m-wide weighing 388 gm per linear meter, impregnated with the per cent brewer's yeast; Size of test strip, 5 cm × 2·5 cm; Number of replicates, 6; Number of larvae exposed 10; Temperature/Humidity, 26·7 ± 0·6°C/68 ± 7 per cent.

† One to two larvae moved away from the fabric during exposure period.

Tables III and IV. The data have been statistically analysed (Tables V and VI). It will be observed that the results do not differ significantly in the different sets of experiments. This provides the requisite confirmation in regard to the concordance in results.

Environmental Conditions

It is essential to maintain temperature and humidity constant during the assessment of the efficiency of insectproofness, since larval activity is influenced [5,6] by variations in temperature and humidity. The choice of suitable temperature and humidity will have to be based on knowledge of the biology of the species of insects concerned. According to Meeuse[7] assessment with moth grubs is carried out at 24 ± 1°C and 60 ± 5% RH and with the larvæ of *A. piceus* at 28 ± 1°C and 60 ± 5% RH. The American test[10] with both moth and beetle larvæ is carried out at 29·5 ± 1·8°C and 60 ± 5% RH. In the Swiss method[9] assessment is carried out at 24 ± 1°C and 60 ± 5% RH using larvæ of *T. bisselliella* whereas in U.K., Germany, Switzerland and Netherlands[8] tests with *T. bisselliella* are carried out at 23-25°C and 65-70% RH. From the knowledge gained on the biology of *A. flavipes*[5] and *T. pellionella*[6], 30 ± 0·5°C with 75 ± 2% RH and 27 ± 0·5°C with 90 ± 2% RH were selected respectively for the two insects, in the insectproofness test method developed in this laboratory.

TABLE III

RESULTS OBTAINED IN DIFFERENT SETS OF EXPERIMENTS* WITH THE SAME AGE OF THE LARVAE OF *A. flavipes*.

Number of sets	Loss in weight (mgm)	Loss in weight (per cent)	Weight of excrement (mgm)	Survival of Larvae (per cent)	Visual Damage
1	74·9	18	37·0	96	Severe
	82·1	18	38·0	92	
	91·3	22	34·6	100	
	100·6	27	48·2	100	
2	95·4	22	52·2	96	Severe
	126·0	28	57·8	100	
	114·4	28	44·2	96	
3	80·6	20	34·6	100	Severe
	111·5	29	41·6	96	
	96·8	25	41·8	96	
4	84·4	21	39·4	100	Severe
	117·7	29	51·8	100	
	74·2	20	36·8	100	
5	83·0	22	34·4	100	Severe
	112·0	30	45·0	100	
	75·8	18	51·0	96	
6	92·3	22	38·8	100	Severe
	94·8	22	45·6	100	
7	71·7	19	33·8	96	Severe
	76·4	21	39·0	100	
	69·0	19	29·6	100	
	100·6	20	39·2	100	
8	95·0	21	31·6	100	Severe
	97·6	21	42·4	100	
	87·8	22	40·0	96	
9	125·6	28	38·4	100	Severe
	122·0	30	48·6	96	
	88·8	23	40·0	100	
10	86·3	23	32·8	92	Severe
	106·8	26	49·2	100	
	108·5	28	49·2	96	
11	106·5	25	46·6	100	Severe
	118·6	27	54·2	100	

*All-wool worsted white serge, 1·37m wide, weighing 388 gms per linear meter (untreated); size of test strip, 5 cms × 2·5 cm Age of larvae, 12-14 weeks; Number of larvae exposed, 25; Period of exposure, 15 days; Temperature/Humidity, 28·9 ± 2·8°/72 ± 8 per cent.

TABLE IV

RESULTS OBTAINED IN DIFFERENT SETS OF EXPERIMENTS* WITH THE SAME AGE OF THE LARVAE OF *T. pellionella*.

Number of Sets	Loss in weight (mgm)	Loss in weight (Per cent)	Weight of Excrement (mgm)	Survival of larvae (Per cent)	Visual damage
1	41·3	12	12·2	100	Moderate
	55·4	15	18·8	100	Moderate
	68·5	19	24·6	100	Severe
	51·5	14	12·2	100	Moderate
	55·0	16	19·6	100	Severe
	61·1	18	21·0	100	Severe
2	70·6	15	25·1	100	Moderate
	62·7	13	19·7	100	Moderate
	103·0	23	37·5	100	Moderate
	42·2	8	12·5	100	Moderate
	83·6	18	28·5	100	Moderate
	68·2	14	23·5	100	Moderate

* All-wool worsted white serge, 1·37 m-wide weighing 388 gms per linear meter, impregnated with ten per cent Brewer's yeast; size of test strip, 5·0 cms × 2·5 cms; Age of larvae, 10 days. Number of larvae exposed 10; Period of exposure, 15 days, Temperature/Humidity, 29·4 ± 2·3°C/85 ± 7 per cent.

TABLE V

ANALYSIS OF VARIANCE

(Based on data in Table III)

	Source of Variance	Degrees of freedom	Mean square	F	F value at 1% level of significance
Per cent loss in weight ..	Between sets of exposure	10	24·085	2·33 (N.S.)	3·26
	Error	22	10·333		
Weight of excrement ..	Between sets of exposure	10	96·141	3·22 (N.S.)	3·26
	Error	22	29·820		
Per cent mortality of larvae ..	Between set of exposure ..	10		0·56 (N.S.)	3·26
	Error	22			

TABLE VI

'T' TEST FOR SIGNIFICANCE

(Based on data in Table IV)

	Averages of		S.E. of the difference of means	't' for 10 DF	Value of 't' at 5% level
	Set 1	Set 2			
Per cent loss in weight	15·73	15·27	2·30	0·20 (N.S.)	2·23
Weight of excrement	18·07	24·47	3·98	1·61 (N.S.)	2·23

Types of feeding tests

Three types of feeding tests are known. These are described below :

Life cycle test—In this method, the test fabric is confined in a chamber containing different stages of the insect. Fresh supplies of insects may be added at intervals to maintain infestation. In the test methods described by Clark[15], Jackson[16], Moore[17] and *Anon*[18] the test fabric is removed after a definite period, say 3 months, and examined for damage. While this method simulates practical conditions of infestation and attack, the chief disadvantages are that it is not quantitative and is not rapid.

Free larval test—In the American[10] and Canadian[11] test methods the larvæ are confined in a container along with the material to be tested and thus they are free to feed on any part of the material. At the end of the pre-determined period, the material is examined and the efficiency of the protective treatment assessed in terms of one or more of the effects produced as a result of feeding. This procedure has been followed in the insectproofness test method developed in this laboratory.

Restricted larval test—In the test methods described by Barrit and Hartley[2], the larvæ are confined to a portion of the test fabric as a result of which they have to feed perforce on the upper side of the fabric. The larvæ are unable to reach the lower surface without eating through the sample. The assessment of efficiency is carried out as in the free larval test.

Slabaugh[19] examined the free and restricted larval tests using larvæ of *A. piceus* and found that the free larval test was superior in respect not only of ease of operation but also of reliability of results. Since the conditions of 'free feeding test' are more akin to natural conditions of infestation than those of restricted feeding test' it was decided to adopt the 'free feeding test' in the insectproofness test method developed in this laboratory.

Baiting of test fabric

According to Meeuse[7] the insectproofness test method can be made more rapid by baiting the test fabric with aqueous extract of dry yeast, which serves as an attractant for the larvæ. The Pest Infestation Laboratory, U.K.[20] has however reported that such baiting does not make material difference to the amount of damage to treated or untreated fabrics. Unbaited fabrics are used for the test developed in this laboratory in respect of *A. flavipes*. In the case of *T. pellionella*, the whole fabric impregnated with yeast is employed since yeast has been found necessary for the growth and development of this insect[6].

Evaluation of results

In the free or restricted larval tests described above the fabric at the end of the test has been examined for extent of attack and efficiency of treatment by widely varying methods by different workers. The most important of these methods relate to observations on (*a*) extent of fibre damage [11,16,19,21 – 25] (*b*) weight of excrement [10,19,21,23 – 27] (*c*) loss of weight of fabric [10,11,21,25,27,28 – 32], (*d*) mortality of larvæ [11,17,22,25,28] (*e*) quantity of uric acid excreted by larvæ during test period [33] (*f*) area of surface attacked [20], (*g*) whether naps or surface fibres have been attacked[11] and (*h*) on visual examination of exposed fabric [34,35]. Of these, (*a*) and (*e*) have not gained support and (*d*) would not stand for both stomach and contact poisons. Methods (*f*), (*g*) and (*h*) are open to subjective errors. On the other hand methods (*b*) and (*c*) can be performed rapidly and quantitatively without the results being vitiated by subjective errors. In view of these considerations methods (*b*) and (*c*) in combination with methods (*d*) and (*h*) have been used for standardisation of insectproofness test method, in this laboratory. The test method has been described below :—

TEST METHOD

Materials and Methods

Test insects—The woolly bear, *A. flavipes* and the case-bearing clothes moth, *T. pellionella* drawn from laboratory cultures are used as test insects. The procedures for rearing the insects in the laboratory are described below:—

A. flavipes—The larvæ are reared at 25-30°C and 70-75 per cent relative humidity of 'barrack' blanket, impregnated with a twenty per cent solution in water of a mixture on equal parts of glucose and albumin. For starting a culture, about 200 adult beetles are liberated on a piece of treated blanket in a glass jar, 18 cms height × 13 cms diameter. The adults are removed from the jar after a week and liberated into a second jar and the process is repeated at weekly intervals. In each jar the number of the adults is maintained at the same level by replacing the dead ones by newly emerged adults. Thus 300-400 eggs are obtained per week in each jar and the larvæ from these cultures when 12-14 weeks old are used as test insects.

T. pellionella—The larvæ are reared at 25-30°C and 70-75 per cent relative humidity on all-wool worsted white serge, impregnated with a five per cent dispersion of brewer's yeast in water. For starting a culture, freshly emerged adults are introduced daily in a glass oviposition container, 15×9 cms. diam. covered with round mesh mosquito-netting held in place with a rubber band. Every morning, eggs are removed by inverting the oviposition jar in a glass dish, 15 cm diam × 4 cm., height. Daily, cultures with known number of eggs (150-200) are started in a rearing container having a piece of the treated woollen fabric. Under the above temperature and humidity conditions, the eggs hatch out within 5-6 days. Each culture thus contains about 150-200 larvæ which when 10 days old are used as test insects.

Test specimens—Sets of ten strips, each 5·0 cms × 2·5 cms are cut from widely spaced portions of the test fabric. Similar strips of an all-wool worsted white serge, 1·37 m-wide weighing 388 gms per linear metre (Serge, White, Lining) are used to serve as untreated control. The fabric to be tested against *T. pellionella* is impregnated with five per cent dispersion of brewer's yeast in water.

Procedure—The test is carried out in glass tubes, 10 cms × 4 cms, which are sealed at one end and covered by muslin held in place by suitable rubber bands at the other. The test strips are individually kept in the glass tubes. There are 6 replicates for the treated fabric and a similar number for the untreated control. Four similar specimens of each set are kept, side by side as humidity checks, and the weight of the test strips is corrected for moisture change in the fabric. The strips to be exposed to *A. flavipes* are placed in desicators having 75 per cent RH inside an incubator at $30 \pm 0{\cdot}5°C$ and those to be exposed to *T. pellionella* are placed in desicators having 90 per cent RH inside an incubator at $27 \pm 0{\cdot}5°C$ for 48 hours before the first weighing. Twenty five larvæ of *A. flavipes* or ten larvæ of *T. pellionella* are liberated for 15 days on the test strips. At the end of the test period the strips are freed of all loose material such as larvæ, excrement and cast skins. Observations on (*a*) loss in weight due to larval feeding, (*b*) weight of excrement of the larvæ, (*c*) survival of larvæ and (*d*) visual damage, are recorded.

The loss of weight in miligrams due to actual feeding of test larvæ as adjusted for humidity changes is calculated as follows:

$$L = \frac{AC}{B} - D$$

where L =adjusted loss of weight in miligrams due to larval feeding,
A =average weight of the six test specimens before testing,
B =average weight of the four humidity check specimens before testing,
C =average weight of the four humidity check specimens after testing,
D =average weight of the test specimens after testing

$$\text{The percentage of loss} = \frac{L}{\frac{A \times C}{B}} \times 100$$

when $A \times \frac{C}{B}$ is the corrected weight for humidity.

RESULTS

A fabric is considered insectproof against *A. flavipes* and *T. pellionella* if the visual damage is nil and the per cent weight loss and the weight of the excrement do not exceed two and four miligrams respectively. These target figures are taken into consideration only when the untreated control fabric records minimum of ten per cent loss in weight due to larval feeding and the weight of the excrement of the larvæ is at least 15-20 mgms.

The above insectproofness test method developed in this laboratory, has been satisfactorily employed [36,37] for evaluating the efficiency of a number of chemicals/treatments functioning as protectants against the wool destroying insects, *A. flavipes* and *T. pellionella*.

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