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Potentialities of Natural Dyestuffs as Antifeedants against Varied Carpet Beetle, *Anthrenus verbasci*

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Abstract

We studied damage to wool fabrics dyed with different natural and chemical dyestuffs by the larvae of varied carpet beetle, *Anthrenus verbasci*, as part of a study on the functions of natural dyestuffs. Eight of ten natural dyestuffs showed an antifeeding effect against *A. verbasci*. Strength of the antifeeding effect of natural dyestuffs in a feeding preference test was in the order lac dye, gallnut, catechu, red cabbage, *Cricula* cocoon extract > cochineal, indigo, Amur cork tree extract > chemical dye. *Lithospermi radix* and turmeric were less effective against *A. verbasci*. The damage to dyed fabrics by the insect was not related to the extent of color depth or shade of the dyed fabric. Water-soluble substances having absorption peaks at around 280 nm, commonly were present in the natural dyestuffs except for turmeric. The polyphenols, tannic acid and catechin having absorption peaks at around 280 nm, seemed to be not related with the antifeeding effect. An alkali degummed *Cricula* cocoon sample that had almost all of the cocoon filament sericin removed showed almost the same level of damage by insect feeding as that of natural *Cricula* cocoons.

Discipline: Insect pest
Additional key words: fabric insect pest, extracts of natural pigment, feeding test, feeding preference test, absorption spectra

Introduction

Natural dyestuffs produced by plants and insects have been used not only for dyeing silk but also as coloring agents in food and cosmetic industries. The interest in these natural dyestuffs is increasing because of recently discovered useful functions such as antioxidant effects[19] and antibacterial effects[16,17], in addition to the positive feelings people have about their safety. They have also gained popularity for the sober and elegant shades that they give to fabrics. It is traditionally believed that many of these natural dyestuffs are effective against insect attack and have some medicinal value. There is an old saying that if a garment is wrapped in a yellow wrapping cloth dyed with turmeric or Amur cork tree extract, it would be protected from insect attack. Feeding inhibition of fabric insect pests, black carpet beetle, *Attagenus unicolor japonicus*, and varied carpet beetle, *Anthrenus verbasci*, by buds of clove, *Syzygium aromaticum*, and calyxes of *Cinnamomum obtussifolium* have been examined[1]. Nakajima (1999)[11] studied widely the feeding damages to wool fabrics, which had been dyed in a dyeing system containing natural dyestuffs in the presence of mordanting agents, by larvae of case-making clothes moth, *Tinea translucens*, and webbing clothes moth, *Tineola bisselliella*, and concluded that the sample dyed with plant dyestuff bayberry, *Myrica rubra*, demonstrated the highest antifeeding effect among the dyed wool samples examined.

Although there are several papers[1,8,9,13,14], discussing the antifeeding effect of repellent substances, feeding habit of *A. verbasci* larvae and so on, very few research papers on the antifeeding effect of natural dyestuffs have been reported. Additionally, very few scientific studies have been carried out focusing on the analysis of the antifeeding factors in natural dyestuffs. We, therefore, studied the antifeeding effect of representative natural dyestuffs derived from plants and insects on the beetle. Further, we used the pigment extracted from cocoons of *Cricula trifenestrata* as one of dyestuff from insects. The reasons for using the pigment extract from *Cricula* cocoons are as follows: (1) it is a kind of natural dyestuff of insect origin, (2) as its common name "golden cocoon"

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suggests, it has a beautiful golden color, (3) the pigment content of the cocoon filaments is high, and (4) the structural characteristics and functionality of the pigment and the substances present in the extract are unknown. Also, to identify the substance present in the natural dyestuff solution, we measured adsorption spectra of the extracts from the natural dyestuffs. In the present study, we show the antifeeding effect of many natural dyestuffs on *A. verbasci* and discuss about the antifeeding factors in dyestuffs.

For two polyphenols, tannic acid and cathechin, used as mordanting agents, many researchers have examined their biological activities[18], but not enough basic information is available on their antifeeding effect on the beetle. We therefore also examined the antifeeding effect of these polyphenols.

Materials and methods

1. Wool fabrics

Wool muslin for the JIS Color Fastness Test (JIS L 0803), hand-washed with Monogen (Dai-Ichi Kogyo Seiyaku Co., Ltd.), was used.

2. Natural and chemical dyestuffs and the method for dyeing

(1) Lac dye, gallnut, catechu, red cabbage and *Lithospermi radix*

Extracts from various plants, lac dye, gallnut, catechu, red cabbage, and *Lithospermi radix* (Tanaka Nao Co., Ltd.) were used. These extracts were filtered through a filter paper, a 5 μm micro-filter and a 1.2 μm micro-filter in a row, before being used for dyeing. Each extract at a concentration of 20% o.w.f. (on weight of fiber) was used for dyeing of the wool fabric. The extract was diluted with water in the ratio of 1:50 and adjusted to pH 5–6 with acetic acid. This dilution was used as a dye solution. The wool fabric was dyed in a dye solution at 60ºC for 1 h.

(2) Cochineal, Amur cork tree extract and turmeric

For the preparation of the extracts of insect pigment, cochineal, and the plant pigments, Amur cork tree and turmeric, the cochineal (Tanaka Nao Co., Ltd.) with 20% o.w.f, Amur cork tree (Tanaka Nao Co., Ltd.) with 50% o.w.f. and turmeric (Tanaka Nao Co., Ltd.) with 50% o.w.f. were used. Each dyestuff was mixed with 50 times as much water, boiled for 30 min, and filtered while hot to obtain the first extract. This extraction process was repeated with the residue using the same amount of water to obtain the second extract. The first and second extracts were combined and left standing in a room until its temperature had dropped sufficiently and then filtered through a filter paper, a 5 μm micro-filter and a 1.2 μm micro-filter in a row, as in the case of (1). Warm water was added to the filtrate to make up the weight to 50 times the weight of the wool fabric to be dyed and 3% o.w.f. of acetic acid was added to prepare the dye solution in which the fabric was dyed at 60ºC for 1 h.

(3) Indigo

The wool fabric was dyed in 10 L solution of the Indian indigo deep-dyeing set (Tanaka Nao Co., Ltd.) following the dyeing method recommended by the manufacturer[15].

(4) *Cricula* cocoons

Twenty grams of *Cricula* cocoons were incubated in 1,000 mL of water at 90ºC for 1 h, and then the water was filtrated through a filter paper, a 5 μm micro-filter and a 1.2 μm micro-filter in a row. The pH of the filtrate was adjusted to 4.5 by the addition of acetic acid and the filtrate was used as a dyeing agent. The wool fabric was dyed in it at 85ºC for 1 h.

(5) Chemical dyes

The wool fabric was dyed in a solution containing 5% o.w.f. of the direct dye, C. I. Direct Black 51 (Commercial product name: Kayaku direct fast black conc.) and 30% o.w.f. of anhydrous sodium sulfate at 90ºC for 1 h.

The fabric dyed by each dyestuff was thoroughly washed first with warm water and then with cold water to remove non-absorbed dye and excess chemicals before using in the insect feeding tests.

3. Treatment of wool fabric using tannin and catechin

Wool fabric was treated with 0.01% tannic acid (reagent grade, Wako Pure Chemical Industries, Ltd.) or 0.01% catechin hydrate (D-(+)-catechin hydrate, $C_{15}H_{14}O_6 \cdot xH_2O$, Nacalai Tesque Inc.) at 60ºC for 1 h. Treated fabric was dried and used for the bioassay.

4. Treatments of *Cricula* cocoons

To study the solubility of the dyestuff and other substances present in the *Cricula* cocoons and to study the relationship between the *Cricula* cocoon extracts and the extent of insect feeding damage, *Cricula* cocoons, treated by one of the methods given below, were used for the insect feeding test.

(1) Water treatment

Cricula cocoons were incubated in water at 90ºC for 1 h by the method described above in **2.**(4). The residues of cocoons were dried at room temperature.

(2) High pressure and high temperature water treatment

Cricula cocoons (62.45 g) were treated in water at 120ºC for 1 h followed by 130ºC for 1 h in an autoclave

(MLS-3780, Sanyo Electric Co., Ltd.). The residues of cocoons were dried at room temperature. After this treatment, the weight of the cocoon residues was 52.6 g (15.76% weight reduction).

(3) Ethanol treatment

Cricula cocoons (3.5 g) were incubated for 5 h in 400 mL of ethyl alcohol in a Soxhlet extraction apparatus and the residues of cocoons were dried at room temperature.

(4) Alkali degumming

Cricula cocoons (8.56 g) were packed in a bag, placed in 1,000 mL of 0.12% Na_2CO_3, and degummed at 130ºC for 2 h[2,3]. The residues of cocoons were dried at room temperature. The weight of the cocoons after degumming was 6.41 g (25.1% degumming loss).

The dried cocoon residues derived from each treatment were used for the feeding tests and a part of the residues was treated with water for the investigation of remaining substances. The water extract was prepared from the incubation of 20 g of each dried cocoon residue in 1,000 mL of water at 90ºC for 5 h following the filtration through filter papers, a 5-μm micro-filter and a 1.2-μm micro-filter in a row. Each water extract was used for the analysis of the absorption spectra.

5. Measurement of color depth

For measuring the color depth of the dyed fabric, the spectral reflectance at the wavelength of maximum absorption was measured using a recording spectrophotometer (UV-3100S, Shimadzu Corp.), and K/S was calculated by the Kubelka-Munk equation:

$$K/S = (1 - R)^2/2R - (1 - R')^2/2R'$$

R: Spectral reflectance at maximum absorption wavelength after dyeing.

R′: Spectral reflectance at maximum absorption wavelength before dyeing.

6. Absorption spectra

Absorption spectra of the solutions, extracted from natural dyestuffs, polyphenols (tannic acid and catechin) and the water extracts from *Cricula* cocoons treated in different ways, were measured in the wavelength range of 200–700 nm. The measurements were carried out using a recording spectrophotometer (UV-3001S, Shimadzu Corp.) and concentration of the test solution was adjusted so that the absorbance would be in the range 0–3 Absorbance.

7. Insect

The extent of damage due to feeding by the insect larvae differs depending on the growth stage and size of the larvae and the food that the larvae were feeding on before the test[10,13]. The following procedure was adopted to carry out experiments on insect attack with high reproducibility and universality of the results. *A. verbasci* larvae found in a cotton bag were collected and reared at 22–24ºC under 23–26% relative humidity (R. H.). Dried and powdered silkworm pupa were used as the rearing food for the successive generations of insects because it had been found earlier that larvae grew well and the mortality was low. Third to 5th-instar young larvae (body length 4.0–5.0 mm and width 1.0–1.5 mm) were selected and used in the feeding tests[4,12].

8. Bioassay

(1) Feeding test

The fabric dyed or treated was cut into 3 × 1.5 cm pieces (the weight of each fabric was about 0.5 g) and placed in a petri dish (9 cm in diameter × 2 cm in height) with 10 *A. verbasci* larvae. There were 5 replicate petri dishes of each type of dyed or treated fabric. The petri dishes were kept in an incubator (22–24ºC, 23–26% R. H.) for 4 weeks. Undyed wool fabric was used as the control.

Damage by the insect was determined by weighing the residual amount of the fabric sample in a balance (BP310S, Sartorius AG). Small pieces of fiber that had detached from the fabric sample by feeding of insects were included in the insect-fed fraction, because they did not retain the shape of the fiber and were difficult to weigh.

Fig. 1 shows the condition of wool fabrics and larvae 4 weeks after the beginning of a feeding test.

(2) Feeding preference test

Eleven pieces of fabric dyed with different dyestuffs were placed along with 100 *A. verbasci* larvae in a 14 cm in diameter × 2.5 cm in height plastic petri dish. Three replicate dishes were placed in the incubator (22–24ºC, 23–26% R. H.). At the time when one of the samples was almost eaten away, the weight reduction of the fabric samples was determined and expressed as the mean values[8–10].

(3) Feeding test on *Cricula* cocoons with different treatments

A *Cricula* cocoon sample of 0.05–0.06 g, treated with one of the methods mentioned above was placed in a petri dish with 10 larvae of *A. verbasci* and kept in an incubator. There were 5 replicate petri dishes of each *Cricula* cocoon sample. Four weeks after the start of the feeding, damage by the insect was determined by weighing of the residual amount of the cocoon sample.

9. Statistical analysis

Data from the feeding test and feeding preference

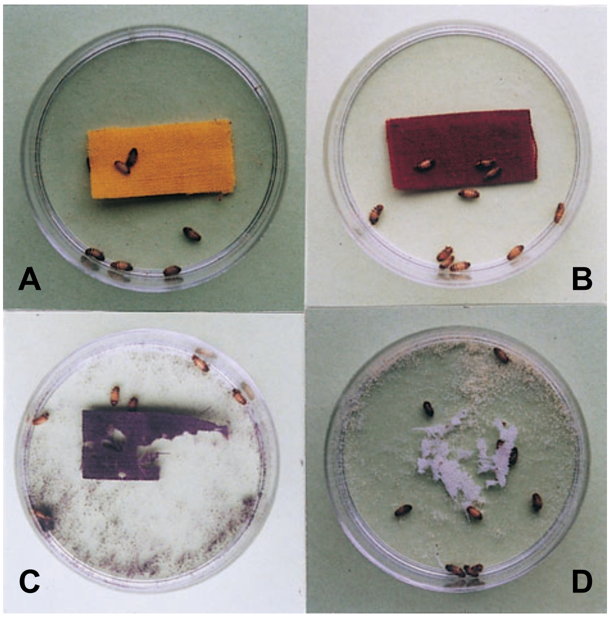

Fig. 1. Wool fabrics and larvae 4 weeks after the beginning of the feeding tests

A piece of dyed wool fabric was kept for 4 weeks with 10 *A. verbasci* larvae.
A: *Cricula* cocoon extract, B: lac dye,
C: *Lithospermi radix,* D: undyed fabric (control).

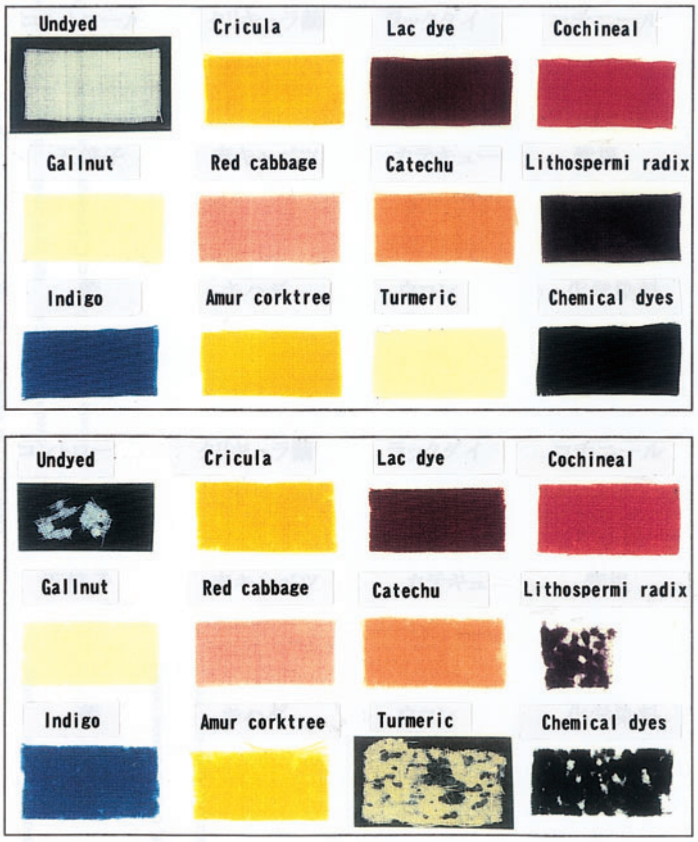

Fig. 2. Damage to wool fabrics dyed with natural dyestuffs by *Anthrenus verbasci* larvae 4 weeks after the beginning of the feeding test

Top: fabric samples before the test.
Bottom: after 4 weeks with *A. verbasci* larvae.

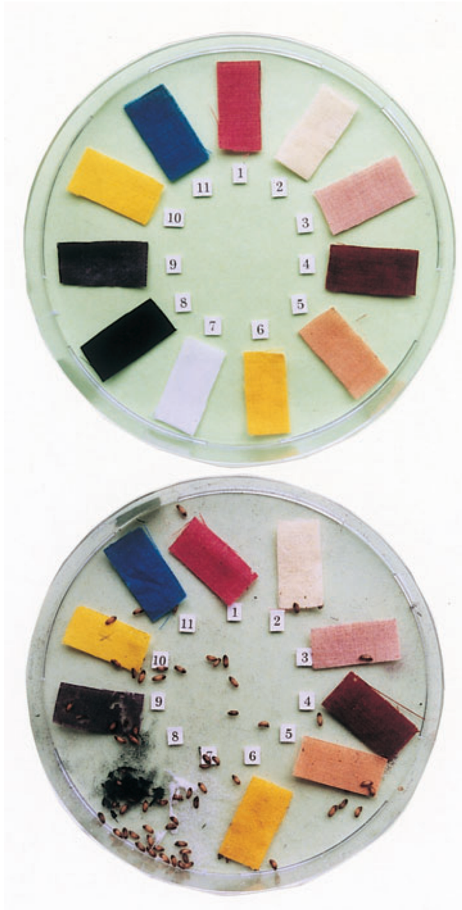

Fig. 3. Result of feeding preference test of *Anthrenus verbasci* larvae to fabrics dyed with natural dyestuffs

Eleven pieces of dyed wool fabric were kept for 3 weeks with 100 *A. verbasci* larvae.
Top: fabric samples before the feeding by *A. verbasci* larvae.
Bottom: 3 weeks after the beginning of the test.
Following natural dyestuffs were used for staining of fabrics.
1: cochineal, 2: gallnut, 3: red cabbage,
4: lac dye, 5: catechu,
6: *Cricula* cocoon extract,
7: undyed fabric (control), 8: chemical dye,
9: *Lithospermi radix,*
10: Amur cork tree extract, 11: indigo.

test were analyzed by using the statistical macro-program, Start 123/win, of Lotus 1-2-3, computer software[20].

Results and discussion

1. Effect of the natural dyestuffs on the feeding of the *A. verbasci* larvae

(1) Feeding test and feeding preference test

The remaining dyed wool fabrics 4 weeks after the beginning of the feeding test, are shown in Fig. 2. Fig. 3 is the results of the feeding preference test for 3 weeks. In the case of turmeric, the dyed fabric was applied only to the feeding test and not to the preference test.

The feeding test and the feeding preference test showed a very similar damage pattern. There were significant differences in the extent of damage among the samples. In both tests, the undyed fabric was attacked first by the beetles, and they were almost completely consumed. In the feeding test, the pieces of wool fabrics dyed with *Lithospermi radix* and turmeric also got extensive damage and lost their original shape. The fabric sample dyed black with the chemical dye developed a number of holes, both large and small. On the other hand, the samples dyed with red cabbage, catechu, indigo, and Amur cork tree extract received only slight insect damage, although they had some small holes. In those dyed with extracts of *Cricula* cocoons, lac dye, cochineal and gallnut, gathering of larvae on the fabric was observed (Fig. 3, bottom), but almost no holes or trace of a hole produced by attack of the insect could be seen.

The results of the damage of dyed wool fabrics by the insect are summarized in Table 1. Values of the damage are given as the means of the amount of consumed wool fabric of five replicates for the feeding test and of three replicates for the feeding preference test, respectively. The extent of the damage measured coincided with the observations mentioned above. In the feeding test, the highest value of the damage was observed for the undyed fabric (fabric before dyeing). This was followed in the order, *Lithospermi radix* > turmeric > chemical dye > Amur cork tree extract > cochineal. The fabric samples dyed with lac dye, gallnut, red cabbage and catechu received as little as 1–2% damage. In the feeding preference test, the fabrics were consumed in the order, undyed fabric (control sample) > chemical dye > *Lithospermi radix* > indigo > cochineal > *Cricula* cocoon extract.

Next, we calculated the significant difference on amount of fed wool fabric in the feeding test by statistical analysis[20] according to both Student's t-test and Fisher's protected least significant difference (Fisher's PLSD). As shown in Table 1, the amount of consumed fabric of most wool fabrics dyed with natural dyestuffs, except for

Table 1. Effect of the natural dyestuffs on the feeding of the *Anthrenus verbasci* larvae

Dyestuffs used for dyeing of fabrics	Amount of consumed wool fabric[a)] (mg) mean ± SD	
	Feeding test (n = 5)[b)]	Feeding preference test (n = 3)[c)]
Lac dye	0.2 ± 0.4 ** (1.7)	1.1 ± 0.3 ** (2.2)
Gallnut	0.3 ± 0.5 ** (1.5)	0.7 ± 0.3 ** (1.4)
Catechu	0.8 ± 0.4 ** (1.5)	0.5 ± 0.3 ** (1.1)
Lithospermi radix	26.1 ± 4.3 (52.6)	13.1 ± 3.3 * (27.2)
Red cabbage	0.2 ± 0.4 ** (0.6)	0.9 ± 0.5 ** (1.9)
Chemical dye	10.4 ± 2.9 ** (21.1)	21.1 ± 9.7 (41.9)
Turmeric	21.5 ± 1.5 * (36.8)	NT
Cochineal	1.0 ± 0.3 ** (2.1)	2.2 ± 0.9 ** (4.4)
Amur cork tree extract	2.2 ± 1.1 ** (4.8)	0.9 ± 0.4 ** (2.0)
Cricula cocoon extract	0.7 ± 0.3 ** (1.4)	1.8 ± 0.7 ** (3.8)
Indigo	0.8 ± 0.4 ** (1.7)	4.4 ± 2.3 ** (8.8)
Undyed fabric (control)	31.6 ± 8.6 (67.7)	39.1 ± 8.6 (81.2)

a): Amount of consumed wool fabric (mg) = (Initial wt. of a sample) – (Final wt. of a sample).
b): 10 larvae of *A. verbasci* were used per one piece of fabric.
c): 100 larvae of *A. verbasci* were used.
Asterisks show the significant difference between dyed fabrics and undyed fabric;
* $P < 0.05$, ** $P < 0.01$ by Student's t-test.
Values in parentheses are mean percentage of damage [(Final wt. of sample/Initial wt. of sample) × 100].
NT: not tested.

Table 2. The multiple comparison among means of the amount of consumed fabric of each dyed fabric in the feeding test[a)]

Dyestuffs used for dyeing fabrics	Lac dye	Gallnut	Catechu	Red cabbage	*Cricula* c. extract	Cochineal	Indigo	Amur cork t. extract	Chemical dye	Turmeric	*Lithospermi radix*	Undyed (control)	Total amount of fed fabric (mg)
Lac dye		–0.12	–0.38	0.08	–0.28	–0.64	–0.44	–1.82	–10.04	–21.12	–25.68	–31.22	1.9
Gallnut			–0.26	0.2	–0.16	–0.52	–0.32	–1.7	–9.92	–21	–25.56	–31.1	2.5
Catechu				0.46	0.1	–0.26	–0.06	–1.44	–9.66	–20.74	–25.3	–30.84	3.8
Red cabbage					–0.36	–0.72	–0.52	–1.9	–10.12	–21.2	25.76	–31.3	1.5
Cricula c. extract						0.36	–0.16	1.54	9.76	20.84	25.4	–30.94	3.3
Cochineal							0.2	–1.18	9.76	20.84	25.04	–30.58	5.1
Indigo								1.38	9.6	20.68	25.54	–30.78	4.1
Amur cork t. extract									8.22	19.3	23.86	–29.4	11
Chemical dye	S	S	S	S	S	S	S	S		–11.08	15.76	–21.18	52.1
Turmeric	S	S	S	S	S	S	S	S	S		4.56	–10.1	107.5
Lithospermi radix	S	S	S	S	S	S	S	S	S	S		–5.54	130.3
Undyed (control)	S	S	S	S	S	S	S	S	S	S	S		158

a): The significant differences among means, shown in the center column in Table 1, were analized by Fisher's PLSD.
Values in upper-right part of the table are difference of two means of the amount of consumed wool fabric dyed with different dyestuffs.
S: Significantly different combination ($P < 0.05$; the absolute value of the difference of the two means larger than 4.215 is significantly different).

those of *Lithospermi radix* and turmeric, were recognized to be highly significant in the difference from the control ($P<0.01$ by Student's t-test). Similar results were obtained in the feeding preference test (Table 1). These results suggest that eight of the ten natural dyestuffs act as strong antifeedants on *A. verbasci*. Further, Fisher's PLSD was used for the multiple comparisons among means of the amount of consumed fabric of each dyed fabric in the feeding test. Results of the analysis are shown in Table 2. The strength of the feeding deterrent effect of 11 kinds of dyestuffs on *A. verbasci* was in the order: lac dye, gallnut, catechu, red cabbage, *Cricula* cocoon extract > cochineal, indigo, Amur cork tree extract > chemical dye > turmeric > *Lithospermi radix*. Wool fabrics dyed with *Lithospermi radix* and turmeric were easily attacked by *A. verbasci* larvae.

The above results show that many of the natural dyestuffs used in the experiments have a significant feeding inhibitory efficacy against *A. verbasci* larvae, a fabric pest, except for *Lithospermi radix* and turmeric.

The repellent effects of the medical herbs, *Syzygium aromaticum* and *Cinnamomum sieboldaii*, which have been traditionally used for insect proof of textile, against varied carpet beetles[7] larvae are known[1]. In this study, we clarified that many natural dyes of plant origin act as antifeedants to varied carpet beetles. In addition, dyes of insect origin also showed a similar effect.

The noteworthy point in the above results is that the extent of insect feeding damage of dyed fabric was not related to the color depth or the shade (Table 3). It is thought that the action of the natural dyestuffs and the medical herbs on the varied carpet beetle is different because the dyestuffs do not have an insecticide action as do the medical herbs which cause the insect's death[2].

(2) Analysis of the absorption spectra of dye solutions

We would like to research further the factors in the natural dyestuffs relative to the feeding inhibitory efficacy. As mentioned above, the extent of damage caused by feeding of *A. verbasci* larvae was not related to the extent of color depth or shade of the dyed fabric. It seems that the food selection of *A. verbasci* larvae is not related with visual stimulus, but with chemical stimulants or inhibitors. To identify the substances present in the dye solutions, the absorption spectrum of each dye solution used was measured in the wavelength range of 200–700 nm (Figs. 4–6).

Fig. 4 is the absorption spectrum of the dye solution obtained after 1 h extraction of *Cricula* cocoons in 90ºC water. The spectrum shows an absorption peak around 280 nm, in addition to the main peak at 430 nm arising from the extracted yellow pigment. When *Cricula* cocoons were extracted in ethyl alcohol at 60ºC for 5 h, no yellow pigment at all was extracted and the extract showed no peak at 280 nm either. From these results, it was concluded that the yellow pigment extracted from *Cricula* cocoons and the substance that showed absorption at 280 nm were both soluble in water but not soluble in alcohol.

Fig. 5 shows the absorption spectra of lac dye, gallnut, catechu, *Lithospermi radix* and red cabbage. Fig. 6 shows the absorption spectra of the chemical dye, turmeric, cochineal and Amur cork tree extract. Absorption peaks around 280 nm could be seen in almost all the natural dyestuffs, although there were some differences in

Table 3. Absorption spectrum of natural dyestuffs solutions and color depth of the wool fabrics dyed with natural dyestuffs

Dyestuffs used for dyeing fabrics	Feeding deterrent effect[a)]	Color depth K/S value	Nanometers of absorption peak around 280 nm and intensity
Lac dye	+++	24	283, s
Gallnut	+++	0.5	275, s
Catechu	+++	3.1	278, s
Lithospermi radix	+	12.7	278, m
Red cabbage	+++	2.8	281, w
Chemical dye	++	24	ND
Turmeric	+	3.8	ND
Cochineal	+++	7.4	280, s
Amur cork tree extract	+++	4.6	280, m
Cricula cocoon extract	+++	11.5	280, m
Indigo	+++	15.7	NT
Undyed fabric (control)	–	0.04	ND

a): Feeding deterrent effect was concluded by Table 2.
Effect: +++, strong; ++, middle; +, weak; –, not found.
Intensity: s, strong; m, medium; w, weak; ND, not detected; NT, not tested.

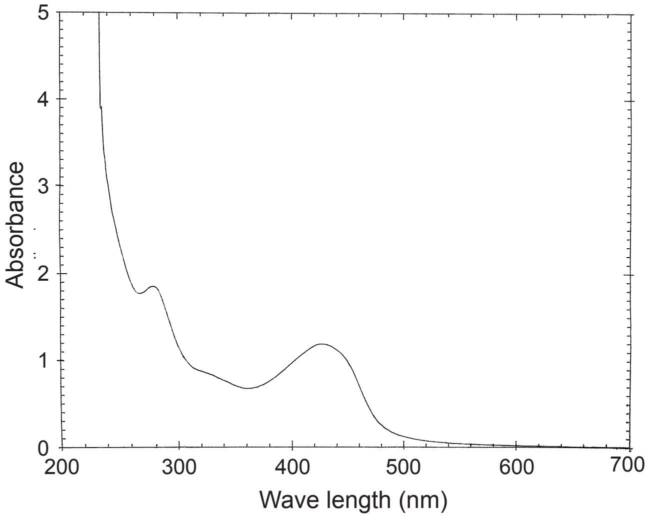

Fig. 4. Absorption spectrum of *Cricula* cocoon extract
Cricula cocoons (20 g) were treated in 1,000 mL of 90ºC water for 1 h.
The extract was then filtered through a 5 μm filter and a 1.2 μm filter.

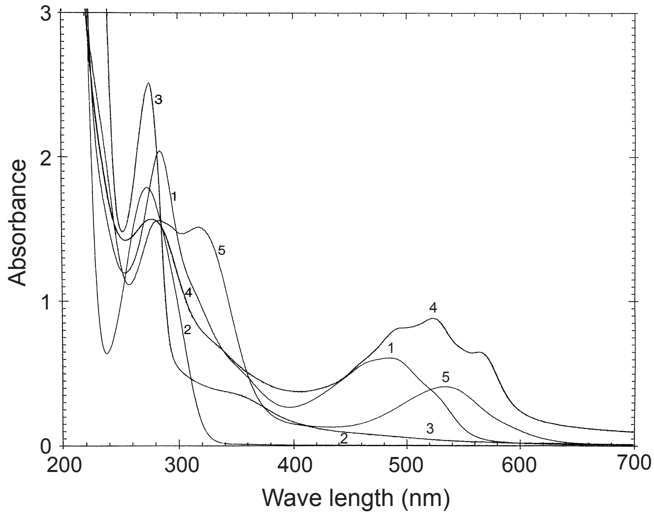

Fig. 5. Absorption spectra of natural dyestuffs – (1)
1: lac dye, 2: gallnut, 3: catechu,
4: *Lithospermi radix,* 5: red cabbage.

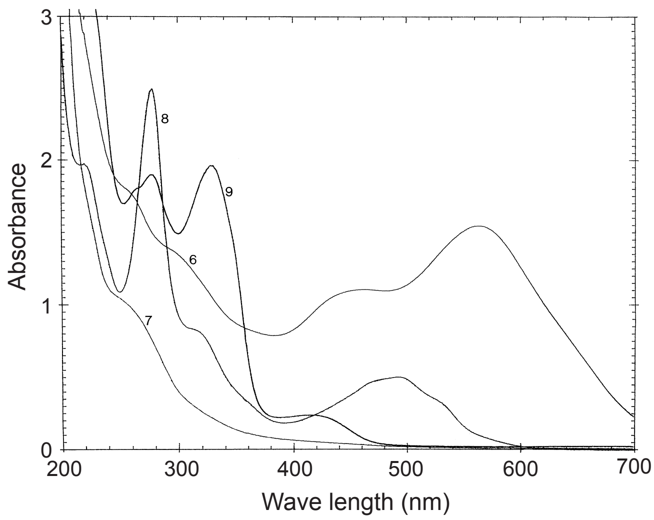

Fig. 6. Absorption spectra of natural dyestuffs – (2)
6: chemical dye, 7: turmeric, 8: cochineal,
9: Amur cork tree extract.
The sample numbers continues from Fig. 5.

intensity. This absorption around 280 nm was more prominent than the absorption caused by the extracted pigments. These results are summarized in Table 3. When we compared the absorption at around 280 nm with the insect feeding damage in each wool fabric dyed with *Cricula* cocoon extract, lac dye, cochineal, gallnut, red cabbage, catechu and Amur cork tree extract, respectively, all of which showed moderate to strong absorption at around 280 nm and had little damage in the wool fabrics dyed with them, showing a clear feeding deterrent effect. On the other hand, the damage was extensive in the wool fabrics dyed with turmeric and the chemical dye, which did not have 280 nm absorption peaks, suggesting the relationship of feeding deterrent effect and absorption at around 280 nm. *Lithospermi radix* was an exception. This dyestuff had a moderately prominent peak around 280 nm but the fabric sample dyed with it showed weak feeding deterrent effect.

The above results suggest that a water-soluble substance with absorption at around 280 nm, which was found commonly in almost all the natural dyestuffs, is a candidate of the factor responsible for the feeding deterrent effect. Red cabbage and Amur cork tree extract had additional fairly prominent absorption peaks at 320–330 nm, where cochineal also showed a low absorption peak. However, we can not throw out the idea that substances having absorption in the spectral regions of 320–330 nm are candidates of the antifeedant.

(3) Substances with strong absorption around 280 nm

We would like to focus mainly on the water-soluble substances with absorption peaks at around 280 nm, which were found in most of the natural dyestuffs. It is obvious that the extract from wild silk cocoons like *Cricula* contains several minor compounds, such as calcium oxalate and polyphenols[5]. The major compound, relating to the insect feeding damage, could be probably polyphenols and not calcium oxalate[6], because calcium oxalate is actually insoluble in water. Then, we measured the absorption spectra for aqueous solutions of commercial products of tannic acid and catechin. Both tannic acid and catechin demonstrated strong absorption peaks at around 280 nm as shown in Fig. 7. The absorption

Table 4. Effect of tannic acid and catechin on the feeding of *A. verbasci* larvae

Treatments	Amount of consumed wool fabric (mg)[a)] mean ± SD (n = 5)	Residual amounts of tannic acid or D- (+)-catechin hydrate[b)]
Not dyed, treated with tannic acid	46.7 ± 5.5 (94.1)	ND
Not dyed, treated with catechin	44.7 ± 3.4 (88.5)	m
Undyed fabric (control)	52.8 ± 2.2 (90.2)	ND

a): Ten larvae of A. *verbasci* were used per one piece of fabric.
Amount of consumed wool fabric = (Initial wt. of a sample) – (Final wt. of a sample).
Values in parentheses are mean percentage of the damage [(Final wt. of sample/Initial wt. of sample) × 100].
b): Residual amounts of tannic acid or catechin within the fabric were estimated from the intensity (m, medium; ND, not detected) of absorption peak at around 280 nm of the soaked solutions got from dyed samples in water at 60ºC for 6 h.

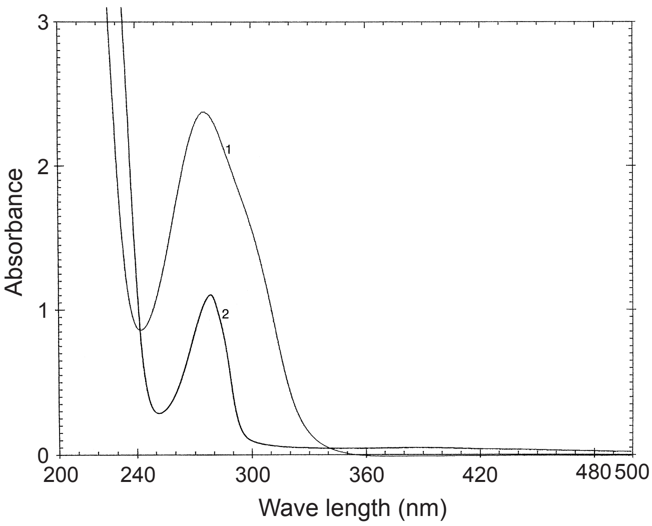

Fig. 7. Absorption spectra for solution of tannic acid and *D*-(+)-catechin hydrate
1: tannic acid (0.05 g/500 mL),
2: catechin hydrate (0.05 g/500 mL).

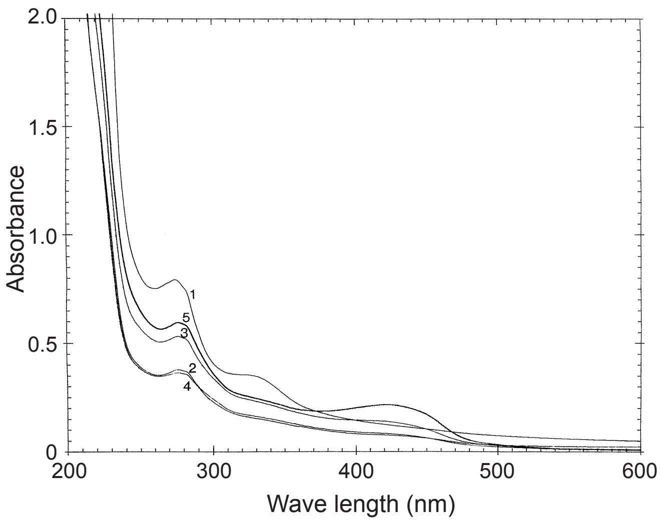

Fig. 8. Absorption spectra of extracts of *Cricula* cocoons treated in different ways
1:alkali-degummed, 2:water-extracted, 3:ethanol-extracted, 4:high-temperature and high-pressure water treatment, 5: no treatment (control).

substances appeared around 280 nm for most of the extracts from natural dyestuffs, and they may be the tannin or catechin of water-soluble polyphenols.

(4) Effects of tannic acid and catechin on the feeding of *A. verbasci* larvae

Effects of tannic acid and catechin on the feeding of *A. verbasci* larvae were examined. The results are shown in Table 4. The wool fabric treated with tannic acid or catechin was less consumed than the control (undyed fabric) by the larvae of *A. verbasci* (Table 4). However, no significant differences on the consumed fabrics were observed between tannic acid or catechin treated fabric and the undyed fabric ($p = 0.05$ by Student's t test). One of the reason that significant differences were not detected may be the low residual amount of both polyphenols within the fabrics (Table 4). At any rate, tannic acid and catechin seem to have weak or no anti-feeding effect.

2. Damage of *Cricula* cocoon filaments by *A. verbasci* with different treatments

From the results of the experiments described above, it is assumed that the substances with antifeeding effect have an absorption peak at around 280 nm. To verify this assumption, we conducted feeding tests on *Cricula* cocoon filaments with different treatments, i.e., water extraction, ethanol extraction, high-temperature and high-pressure water treatment, and alkali degumming. Fig. 8 illustrates the absorption spectra of the extracts of the residues of *Cricula* cocoons in different treatments. The absorption spectra of the extracts in the water system and in hot water at elevated temperature and high-pressure water treatments were similar as shown in Fig. 8. Their intensity of absorbance around 280 nm was slightly weak compared to the extracts of the ethanol extracted and the untreated cocoons. The damage to *Cricula* cocoons also tended to be slightly weak as described in Table 5.

Table 5. Damage to *Cricula* cocoon filaments by *Anthrenus verbasci* larvae with different treatments and absorption intensity of the soakages obtained from dyed samples

Treatments	Details of treatments	Amount of consumed *Cricula* cocoons (mg)[a)] mean ± SD (n = 5)	Intensity of absorption around 280 nm[b)]
Alkali degumming (reference)	Degummed in a 12 g/L sodium carbonate solution at 130ºC for 2 h. (25.1% degumming loss)	9.5 ± 1.9 (18.6)	m
Water extraction	Cocoons extracted in 90ºC water for 1 h.	9.7 ± 1.6 (18.3)	w
Ethanol extraction	Extracted for 5 h in ethanol.	10.2 ± 2.0 (18.8)	m
High temp.-high pressure water treatment	Treated in 120ºC water for 1 h and in 130ºC water for 1 h. (15.8% weight reduction)	7.4 ± 1.4* (13.6)	w
No treatment (control)		9.3 ± 1.2 (18.0)	m

a): Ten larvae of *A. verbasci* were used per one piece of fabric.
Amount of consumed wool fabric = (Initial wt. of a sample) – (Final wt. of a sample).
Values in parentheses are mean percentage of damage by feeding [(Final wt. of sample/Initial wt. of sample) × 100].
* Significant difference between treatments and no treatment (P < 0.1 by Student's t-test).
b): Intensity (w, weak; m, medium) of the soakage immersed out (in water at 90ºC for 5 h) resulting from the residues of *Cricula* cocoons with different treatments.

Table 5 shows the damage of the residues of *Cricula* cocoons with different treatments by *A. verbasci* larvae and absorption intensity of the extracts obtained from each cocoon residue. The alkali degumming sample (Fig. 8-1) that had almost all of the cocoon filament sericin of *Cricula* removed by alkali treatment and had a 25.1% weight loss, showed almost the same level of insect feeding damage as other *Cricula* cocoon residues that still had the cocoon filament sericin. The fact that the insect feeding damage in *Cricula* samples does not relate to the existence of sericin of *Cricula* cocoon filaments is clearly different from the previous results shown for silk fibers from silkworm, *Bombyx mori,* where the damage of degummed silk (consisting of fibroin) was much less than that of raw silk (consisting of sericin and fibroin)[13,14]. We would like to study this aspect further to identify the chemical substances which influence the feeding behavior of the insect.

3. Conclusion

It has been traditionally believed that natural dyes with yellow color like turmeric and Amur cork tree extract (*Phellodendron amurense*) show an antifeeding effect against feeding damage by the larvae of *Attagenus*. In this study, we clarified that many natural dyestuffs show an antifeeding effect against varied carpet beetle for the first time. Additionally, the antifeeding effects of *Syzygium aromaticum* and *Cinnamomum sieboldaii* against varied carpet beetles' larvae of *A. verbasci* and *Attagenus unicolor japonicus* Ratter were reported previously in natural dyeing[1], but we demonstrated that many natural dyestuffs, which are not limited to yellow color, have significant antifeeding effects in feeding tests with *A. verbasci* larvae. Although we could not conclude what are the antifeedants, many natural dyestuffs contain inhibitors of feeding behavior.

The final goals of the present paper describing absorption spectra are to determine precisely the chemical components which might act as the antifeedants of harmful insects and consider deeply the relationship between the effective chemical components and the absorption peak around 280 nm. It should be kept in mind in future that the absorbance at around 280 nm could be due to an overlapping effect by tannic acid, catechin, amino acids, and other minor components present in the extracts from natural products.

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