Repellent effects of Moutan and its extract against several species of stored-product insects in the Chinese medicinal materials

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Abstract

Antagonistic storage is one of the traditional storage ways of the Chinese medicinal materials The results indicate that antagonistic storage between Moutan and Alismatis have obvious effects on insect-repellency and mothproof based on the trials in a warehouse for storing Chinese medicinal materials for six months.

In order to ascertain the mechanism of insect-repellency of Moutan in the antagonistic storage system, the paper strip method described by McDonld et al (1970) and food preference method described by Mondal (1984) were used to test repellency of Moutan extract and Paeonol against five species of stored-product insects in the Chinese medicinal materials The results are summarized as:

The average repellency of 100, 200, 400$\mu g/cm^2$ Moutan extract against five species adults totally achieved Class Ⅴ (80.1 – 100% repellency), and the order of repellent activity of Moutan extract to them was *Rhyzopertha dominica* ≤ *Stegobium paniceum* < *Oryzaephilus surinamensis* ≤ *Sitophilus zaemais* < *Tribolium castaneum*.

Percentage repellency of *S. paniceum* adults was negatively correlated with weeks of treatment with Moutan extract.

The average repellency of 0.25%, 1.25%, 2.5% Moutan extract to *T. castaneum* larvae from the first instar to the sixth instar achieved Class Ⅱ (20.1 – 40% repellency), Ⅲ (40.1 – 60%), Ⅲ, Ⅲ, Ⅳ (60.1 – 80%), Ⅴ, respectively

It is feasible applying the index RC50 comparing the repellent activity of various chemicals against various insect species, and the conclusions are the same as those of the variance analysis

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Introduction

Antagonistic storage is one of the traditional storage ways of the Chinese medicinal materials and it is seldom used on the other commodity storage It mainly utilizes some of the special odorous crude drugs which have repellent effects on insects, together with other crude drugs that are easily damaged by the stored-product insects, and in this way, the aim of preventing insect damage can be realized. This was recorded in the medicinal book 'BENCHAOMONGQUAN' written by Chen Jia-mo as early as Ming Dynasty For example, antagonistic storage between Moutan and Alismatis is commonly used, and if they are stored together, Alismatis will not be infested by insects and the colour of Moudan will not be changed (Liu, C.N., 1987; Kong, W.Y., 1988).

The main components of Moutan are Paeonol, Paeonoside, Paeonolide, Paeoniflorin, benzoic acid, volatile oil and sterol (Xu, G J., 1987). It is held that Paeonol has special perfume odour and strong ability of penetration, and if storing Alismatis together with Moutan, a kind of protective film will be formed on the surface and this film has functions of insect-repellency and sterilization (Liu, H. Z., 1987). But no person has done researches on this assumption till now

The aim of our research is to determine repellent effects of Moutan and its extract against several species of stored-product insects in the Chinese medicinal materials.

Materials and Methods

Test materials

Moutan is the dried root and cortex of *Paeonia suffruticosa* Andr (Ranunculaceae). Alismatis is the dried tuber of *Alisma orientalis* (Sam) Juzep. (Alismataceae) They were supplied by the Nongfeng Bridge Storage Warehouse of the Chinese Medicinal Materials, Beipei District, Chongqing, P. R. China (hereinafter called the Nongfeng Warehouse)

Moutan was ground into fine powders (80 mesh) and then extracted with n-hexane in a Soxhlet's extractor at 70 – 80℃ for 24 hours The extract was concentrated in a rotary evaporator and finally made solvent free in a vacuum desiccator A yellow oily extract (hereinafter called Moutan extract) was obtained The efficiency of extraction was 2.44% by weight of Moutan powders

Paeonol was analytical agent, whose active ingredient is greater than 99%, molecular formula is $C_9H_{10}O_3$, and its molecular weight is 166.17 It was supplied by the Chinese Examination & Determination Institute of Drugs & Biological Products.

Test insects

Stegobium paniceum (L) was reared on *Nelumbo nucifera* Gaertn. (Nymphaeaceae) diet, *Rhyzopertha dominica* (F.) was reared on *Coix lachryma-jobi* L *var. ma-yuen* (Roman) Stapf (Gramineae) diet They were in incubators maintained at 30 ± 1℃ and 65 – 75% relative humidity

Sitophilus zeamais Motschulsky was reared on barley, *Hordeum vulgare* L (Gramineae) diet; *Tribolium castaneum* (herbst) and *Oryzaephilus surinamensis* (L) were reared on barley powders added to 5% yeast They were in incubators maintained at 28 ± 1℃ and 65 – 75% relative humidity.

These insects were collected from the Nongfeng Warehouse.

Antagonistic storage trials

Put Moutan and Alismatis respectively 80 grams per glass jar There were two treating ways, one was covering two drugs layer by layer with Moutan on top; the other was that Alismatis was buried in Moutan. Only Alismatis (no Moutan) served as control Three replicates per treatment. All the jars were uncovered and placed in the Nongfeng Warehouse for six months. Numbers of insects and percentage of bored Alismatis in each jar were recorded at the end of the trials.

Repellency tests

Paper strip method: Repellency of each species adults was tested by the method described by McDonld et al (1970) Each filter paper (8.6cm in diameter) was cut into two halves and one half was dipped with 0.5ml solutions of test chemical in acetone to obtain deposits at various concentrations The other half treated with 0.5ml acetone served as control. After the acetone evaporated, each treated strip was attached lengthwise, edge-to-edge to a control strip with tape on the reverse side A filter paper was put on the bottom of each glass Petri dish (8 6cm in diameter). Twenty unsexed adults (two days old) were then released in the middle of the filter paper and the dish had been laid in an incubator at 28℃ without light The number of adults on each half of the filter paper was recorded once every 12 hours for 3 consecutive days. There were three replicates per treatment The average result of 3-day period for each replicate was converted to percentage repellency and its average was taken

Food preference method: Repellency of *T castaneum* larvae was tested by the method described by Mondal (1984) A filter paper was placed on the bottom of each glass Petri dish (8 6cm in diameter) and one half was covered with a very thin layer of flour medium treated with Moutan extract The other half served as control and was provided with a layer of untreated flour Thirty larvae identified as the same instar were introduced at the centre of the filter paper and the dish was placed in an incubator at 28℃ without light The number of larvae on each half of the filter paper was recorded after one hour There were five replicates per treatment. Percentage repellency was calculated for each replicate and the average was taken.

Calculation of percentage repellency: Percentage repellency was calculated by the method described by Laudani et al. (1955):

$$\text{Repellency} = \frac{C - T}{C} \times 100\%$$

Where T = mean number of insects on treated paper; C = mean number of insects on control paper

The mean repellency for each treatment was assigned to a repellency class according to the standard described by McGovern et al. (1977): Class 0 = < 0.1% repellency; Class I = 0.1 – 20%; Class II = 20.1 – 40%; Class III = 40.1 – 60%; Class IV = 60.1 – 80%; Class V = 80.1 – 100%.

RC_{50} represents the concentration of a chemical when its percentage repellency to insects is achieved by 50%.

Multiple range test

The data obtained were analyzed with Duncan (1955) multiple range test ($p = 0.05$).

Results and Discussion

Effects of insect-repellency and mothproof on antagonistic storage with Moutan and Alismatis

The mean individuals of insects in each jar and percentage of bored Alismatis in two antagonistic ways are significantly lower than the control (see table 1). The results indicate that antagonistic storage with Moutan and Alismatis has obvious effects on insect-repellency and mothproof, and it confirms that the records associated with the antagonistic storage in the book 'BENCHAOMONGQUAN' are correct.

The mean individuals of insects in each jar by two antagonistic ways are not significantly different ($p > 0.05$) (see table 1). The results indicate that two antagonistic ways can be used in the practical antagonistic storage with

Moutan and Alismatis.

Table 1. Effects on insect-repellency and mothproof after antagonistic storage with Moutan and Alismatis for six months

Treating Ways	Mean individuals of insects per jar (include beetles & moths)	Percentage of bored Alismatis (%)
Across layer by layer	4.7 b	0
Burying storage	5.3 b	0
Control	21.3 a	83.3

Means within columns followed by the same letter aren't significantly different ($P>0.05$)

Repellent effects of Moutan extract on five species of stored-product insect adults

Table 2 indicates that the repellent class of 100μg/cm² Moutan extract against *T castaneum* adults is the highest Class V, but at the same concentration it against the other four species of insect adults are much lower. Only when the concentrations is above 200μg/cm² can the repellent effect on the other four species reach to the similar level of that on *T. castaneum*

Percentage repellency between *S. zeamais* and *O surinamensis* or between *S paniceum* and *R dominica* is not significantly different ($p > 0.05$). However, percentage repellency between *T. castaneum* and *S zaemais* or *O. surinamensis* is significantly different ($p < 0.05$) So is percentage repellency between *T castaneum* and *S. paniceum* or *R. dominica* ($p < 0.05$)

The mean repellency of concentration 200, 400, 800μg/cm² Moutan extract against five species adults are of less difference The order of repellent activity of Moutan extract to them is *R dominica* ≤ *S paniceum* < *O. surinamensis* ≤ *S. zaemais* < *T. castaneum*.

Table 2. Percentage repellency of three concentrations of Moutan extract against five species of stored-product insect adults

Insect species	Concentrations of Moutan extract		
	100μg/cm²	200μg/cm²	400μg/cm²
S paniceum c	68.53 c	82.83 c	91.93 bc
S. zeamais b	78.47 b	90.00 b	93 00 b
R. dominica c	72.27 bc	82.73 c	87.97 c
T. castaneum a	92.40 a	96.33 a	98.10 a
O surinamensis b	70.97 c	91.97 b	94.03 b

Means within columns followed by the same letter aren't significantly different ($P>0$ 05)

Regression relationship between repellency of adult insects and concentration of chemical

Table 3 indicates that percentage repellency of adult insects increases with the increase of the concentration of Paeonol or Moutan extract. If repellency on adult insects adults is required to reach to Class V, the concentrations of Moutan extract against adults of *S. paniceum* & *O. surinamensis* need respectively above 200μg/cm², the concentration of Paeonol against *S paniceum* adults needs above 100μg/cm²

Based on the data of table 3, the regression equations between repellency probability of adult insects (Y) and logarithm of concentration (X) are developed as:

Regression equation of Paeonol to *S paniceum* adults.

$Y=3.2617+1.3629X$ ($r=0.9836^{**}$)

$RC_{50}=18.86\mu g/cm^2$ (see figure 1A)

Regression equation of Moutan extract to *O surinamensis* adults.

$Y=3.1385+1.3421X$ ($r=0.9769^{**}$)

$RC_{50}=24.38\mu g/cm^2$ (see figure 1B)

Regression equation of Moutan extract to *S. paniceum* adults.

$Y=3.0251+1.3550X$ ($r=0.9798^{**}$)

$RC_{50}=28.67\mu g/cm^2$ (see figure 1C)

Figure 1 indicates that line A, B and C are nearly parallel, the repellent activity of various chemicals against various insect species can be compared with the RC_{50}. The less RC_{50} value, the higher the repellent activity of the chemical. The order of RC_{50} value was $A < B < C$ Therefore, the minimum level of repellent activity increases in the following order: Moutan extract to *S paniceum* < Moutan extract to *O. surinamensis* < Paeonol to *S paniceum*. The conclusions are the same as those of the variance analysis. It is confirmed that it is feasible to compare the repellent activity of various chemicals against various insect species with the index RC_{50}.

Table 3. Percentage repellency of seven concentrations Paeonol and Moutan extract against adults of *S. paniceum* and *O. surinamensis*.

Concentrations (μg/cm²)	Paeonol	Moutan extract	
	S. paniceum	*S paniceum*	*O surinamensis*
25	63.97 e	54.07 e	57.03 e
50	67.93 e	63.90 d	62.53 e
100	81.83 d	69.20 d	71.03 d
200	90 90 c	83.60 c	92.40 c
400	95.13 b	93.47 b	94.30 bc
600	98.10 a	95.90 ab	96.27 ab
800	99.23 a	98.30 a	98.03 a

Mean within columns followed by the same letter aren't significantly different ($P>0$ 05)

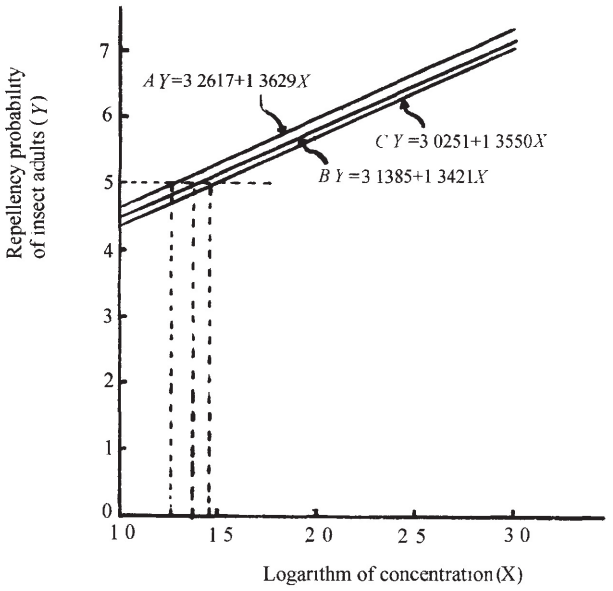

Fig. 1. Regression relationship between repellency probability of insect adults and logarithm of concentration

Regression relationship between percentage repellency of S. paniceum adults and weeks after treatment with Moutan extract

Table 4 indicates that percentage repellency of *S paniceum* adult decreases with the increase of weeks after treatment with Moutan extract Repellent effect of Moutan extract on *S. paniceum* adults, at 200μg/cm² concentration decreases to Class Ⅳ and Class Ⅲ respectively from the third week to the seventh week and the eighth week; at 400μg/cm² concentration decreases to Class Ⅳ, from the sixth week to the eighth week; at 800μg/cm² concentration still keep Class Ⅴ in the eighth week

The mean repellency of 200, 400 and 800μg/cm² concentrations Moutan extract against *S. paniceum* adults, keeps Class Ⅴ from the first week to the fifth week, decreases to Class Ⅳ from the sixth week to the eighth week. The mean repellency between the first week and the second or the third week, between the third week and the fourth week, between the seventh week and the eighth week were not significantly different ($p>0.05$)

Based on the data of table 4, the regression equations between percentage repellency of *S. paniceum* adult (Y) and weeks after treatment with Moutan extract (X) are developed as:

Regression equation of 200μg/cm² Moutan extract

$Y=88.2421-3.3713X$ ($r=-0.9393^{**}$) (see figure 2 A)

Regression equation of 400μg/cm² Moutan extract.

$Y=97.1132-3.0465X$ ($r=-0.9440^{**}$) (see figure 2 B)

Regression equation of 800μg/cm² Moutan extract.

$Y=101.0750-2.1083X$ ($r=-0.9834^{**}$) (see figure 2 C)

Figure 2 indicates that percentage repellency of *S. paniceum* adults is negatively correlated with weeks after treatment with Moutan extract The comparison of the declining speeds of percentage repellency of various concentrations of Moutan extract to *S. paniceum* adults, indicates that at 200μg/cm² concentration it decreases the fastest, at 800μg/cm² concentration the decrease is the slowest, and at 400μg/cm² concentration it decreases medially The results indicate that the lower the concentration of Moutan extract, the faster the declining speed of percentage repellency to *S. paniceum* adults

Table 4. Percentage repellency of three concentrations of Moutan extract against *S. paniceum* adults at various weeks after treatment

Time (weeks)	Concentrations of Moutan extract		
	200μg/cm²	400μg/cm²	800μg/cm²
1 a	81.20 a	91.07 a	97.70 a
2 a	80.23 ab	90.23 ab	97.10 a
3 ab	79 60 ab	89.23 ab	95.93 ab
4 b	78.77 ab	87.80 ab	93.17 bc
5 c	74.13 bc	84.97 b	91.20 cd
6 d	70.4 7 c	77.30 c	87.80 de
7 e	61.40 d	77.53 c	84.97 e
8 e	58 77 d	69.10 d	84.83 e

Mean within columns followed by the same letter aren't significantly different ($P>0$ 05)

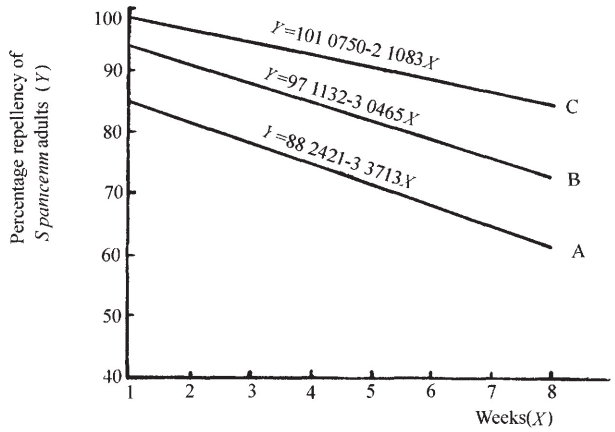

Fig 2. Regression relationship between percentage repellency of *S paniceum* adults and weeks after treatment with Moutan extract

Repellent effects of Moutan extract against various instars of *T. castaneum* larvae

Table 5 indicates that the repellent activity of Moutan extract to *T castaneum* larvae increases with increasing

of the instar of *T castaneum* larvae For example, for repellency class of 1.25% Moutan extract to *T castaneum* larvae, the first and second instars only reach Class Ⅱ, the third instar may reach Class Ⅲ, the fourth and fifth instars may reach Class Ⅳ, the sixth instar can reach Class Ⅴ. Another example is repellency class of 2.5% Moutan extract to *T. castaneum* larvae, the first instar only reaches Class Ⅱ, the second and third instars may reach Class Ⅲ, the fourth instar may reach Class Ⅳ, the fifth and sixth instars can reach Class Ⅴ. The results indicate that repellent reaction of the lower larval instars of *T castaneum* to Moutan extract is less sensitive, but that of the higher larval instars is more sensitive

The mean repellency of 0.25%, 1 25% & 2.5% Moutan extract to *T. castaneum* larvae, between the second instar and the third instar, between the fifth instar and the sixth instar is not significantly different ($p>0.05$) However, the mean repellency between the first instar and the second or the third instar, between the fourth instar and the fifth or the sixth instar is significantly different ($p<0.05$).

The mean repellency of 0.25%, 1.25% & 2.5% Moutan extract to *T. castaneum* larvae from the first instar to the sixth instar achieve Class Ⅱ, Ⅲ, Ⅲ, Ⅲ, Ⅳ, Ⅴ, respectively Therefore, repellent activity of Moutan extract against *T. castaneum* larvae increases instar by instar

Table 5. Percentage repellency of three concentrations of Moutan extract to various instars of *T castaneum* larvae

Larval instars	Concentrations of Moutan extract		
	0.25%	1.25%	2.5%
First d	21.06 c	26.98 d	30.70 d
Second c	36.44 b	38.20 c	49.22 c
Third c	44.92 b	48.26 c	49.84 c
Fourth b	43.50 b	60.78 b	66.56 b
Fifth a	64.36 a	78.70 a	83.48 a
Sixth a	70.36 a	83.62 a	86.40 a

Mean within columns followed by the same letter aren't significantly different ($P>0$ 05)

Conclusions

Clearly, Moutan extract has remarkable repellent activity against several species of main stored-product insects in the Chinese medicinal materials based on the laboratory bioassays Insect-repellent effect of Moutan in the antagonistic storage system between Moutan and Alismatis is undoubtedly correlated with the repellent activity of Moutan extract against stored-product insects. Moutan extract has many components of repellent activity, in which Paeonol is the main component of repellent activity. It is confirmed by the fact that the repellent activity of Paeonol to *S. paniceum* adults is higher than that of Moutan extract. It needs further study about what the structures of the other repellent components in Moutan are and what their repellent activities are.

Faced with the reality that the stored-product insects increasingly infest with the Chinese medicinal materials and the resistance of the stored-product insects is caused by the application of fumigants, the further studies on the mechanisms of insect-repellency in the antagonistic storage of the Chinese medicinal materials may be helpful to understand the inherent characters of the antagonistic storage and it is significant to store different varieties of crude drugs properly and control the stored-product insects by the application of own ingredient chemicals on the integrated management of the stored-product insects in the Chinese medicinal materials

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