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FULL LENGTH ARTICLE

Evaluation of the toxicological effect of bean flour on the mortality and population dynamics of two storage mites

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Abstract A laboratory study was carried out to demonstrate the efficiency of bean flour in the control of two storage mites: *Tyrophagus putrescentiae* (Schrank, 1781) and *Aleuroglyphus ovatus* (Troupeau, 1878) fed on two dried Chinese herbs: coix seed (*Coix lachrymal-jobi*) and Chinese hawthorn (*Crataegus pinnatifida*). Five concentrations of bean flour (0%, 0.01%, 0.1%, 1% and 10%) were used at 25 °C and 85 ± 5% relative humidity (RH) under darkness. The results indicated that the controlling effect of bean flour on *T. putrescentiae* was higher than that on *A. ovatus* and became more noticeable when its concentration was increased. Low dose of bean flour (0.01%) generally had no toxic effect on *T. putrescentiae* and *A. ovatus* when they fed on *C. lachrymal-job* and *C. pinnatifida*. Mortalities of *T. putrescentiae* on *C. pinnatifida* varied from 15% to 40% and from 26% to 61% on *C. lachrymal-job* due to the use of 0.1% of bean flour. Moreover, in the case of *A. ovatus*, the mortalities ranged from 6% to 26% and from 15% to 51% on *C. pinnatifida* and *C. lachrymal-job*, respectively. After 28 days, the concentration of 1% bean flour caused 81% and 52% mortalities of *T. putrescentiae* and *A. ovatus* on *C. pinnatifida* and 92% and 69% on *C. lachrymal-job*. Addition of bean flour at 10% on *C. pinnatifida* and *C. lachrymal-job* killed all individuals of *T. putrescentiae* and *A. ovatus*.

The results showed also that population dynamics of *T. putrescentiae* and *A. ovatus*, which is explained by the rate of increase (*r* values) on *C. pinnatifida* and *C. lachrymal-job* did not change considerably at 0% and 0.01% concentrations of bean flour. On the other hand, *r* values of *T.*

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putrescentiae and *A. ovatus* reread on *C. pinnatifida* and *C. lachrymal-job* sharply decreased at other concentrations of bean flour (0.1%, 1% and 10%) after 21 days. It can be concluded that 1% of bean flour is a good concentration to control *T. putrescentiae* after 28 days on *C. pinnatifida* and *C. lachrymal-job*, but it is not good enough in the case of *A. ovatus*.

1. Introduction

Storage mites (Acari: Astigmata) are harmful pests and greatly distributed in the countries, which are characterized by high humidity climate (Hubert and Pekár, 2009). There are many reports which showed that storage mites such as *Tyrophagus putrescentiae* (Schrank. 1781) and *Aleuroglyphus ovatus* (Troupeau, 1878) can affect various stored products, including flour, dried fruits, vegetables, grains, seeds, and ornamental and medicinal plants (Hughes 1976; Hubert and Pekár, 2009; Stará et al., 2011; Hubert et al., 2013). Attack of storage mites can cause huge losses in the quality and safety of stored products due to the accumulation of fungi mycotoxins and mite residues, which are derived from their activities (Hubert et al., 2003; Hubert et al., 2004; Nesvorna et al., 2012). Moreover, these mites can also lead to hazardous health problems for humans such as allergens, respiratory disease and atopic dermatitis (Franzolin et al. 1999; Colloff, 2009). It was recorded by Hubert et al. (2013) that the storage mite threats are whispered to be serious when their population densities are high (> 1000 mites/kg stored product).

There are many methods that have been applied to control storage mites. The use of physical techniques, which generally depends on the changing of temperature and humidity of dried products stores, faced some limits to apply them. For example, the farmers cannot professionally manage the temperature and humidity, and this may cause high increases in the population of storage mites (Hubert et al., 2006a). Chemical control of storage mites using different acaricides is an effective and economical method but they havelegal limits due to their harmful effects on human health (Sanchez-Ramos and Castanera, 2001; Collins, 2006). Moreover, the resistance of storage mites can be increased as a result of continuous addition of chemical pesticides (Zettler and Arthur 2000; Hubert et al., 2007). For that reason, it is very important to investigate good and suitable methods to control and decrease the population of storage mites without causing harmful effects on humans.

Recently, many investigations have been focused on the development of new natural pesticides to control stored product pests. For example, botanical pesticides such as protein-enriched pea flour (*Pisum sativum*) showed high efficiency in controlling stored grain pests (Hou and Fields 2003; Fields 2006). It was mentioned by Hubert et al. (2006) and Hubert et al. (2007) that the application of bean flour (*Phaseolus vulgaris*) caused significant decreases in the population of storage mites. The effect of legume flour has been well evaluated on stored product insects (Fields et al. 2001; Hou and Fields 2003; Fields 2006). However, few studies were conducted to evaluate the toxic potential of bean flour (*P. vulgaris*) as an acaricide on storage mites (Hubert et al., 2006; Hubert et al., 2007; Hubert and Pekár, 2009). To the best of our knowledge, no study has been done to examine the efficiency of bean flour as an antifeedant against storage mites, which infested dried Chinese herbs. Therefore, the current study was conducted to estimate the toxic influence of different bean flour doses on the mortality and population dynamics of two storage mites (*T. putrescentiae* and *A. ovatus*) fed on two dried Chinese herbs (*Crataegus pinnatifida* and *Coix lachrymal-jobi*).

2. Materials and methods

2.1. Bean flour and Chinese herbs

The bean flour (*P. vulgaris* L.), which was used as an antifeedant in the current study was obtained from the College of Horticulture at Huazhong Agricultural University (HZAU), Wuhan, China. Seeds of bean were dried at 50–60 °C for 48 h, ground to powder with an electric mill, and then stored in a polyethylene bag for future usage. Two Chinese herbs, coix seed (*C. lachrymal-jobi*) and Chinese hawthorn (*C. pinnatifida*) were also collected from the College of Plant Science and Technology at HZAU. The herbs were dried at 50–60 °C for 48 h and then crushed to small pieces to use them as diets for storage mites.

2.2. Storage mites

Two stored product mites, *T. puterscentiae and A. ovatus* were used in our research and collected from the College of Plant Science and Technology at HZAU. The mites were mass-reared according to the method of Hubert et al. (2006). In brief, *T. puterscentiae* and *A. ovatus* were placed in glass flasks (volume 1000 ml) containing a rearing diet which consisted of 45 g oat flakes, 45 g wheat flour and 10 g yeast. After that, the flasks were covered by muslin and kept in an incubator at 25 °C and 85 ± 5% RH in the dark. *T. puterscentiae* and *A. ovatus* were transferred individually to other rearing flasks contained herbs for one week for the acclimation process before beginning the experiment.

2.3. Experimental design

A factorial experiment with three factors (Chinese herbs, storage mites and bean flour concentrations) was set up in a randomized complete block design with ten replicates per treatment. The experiment was carried out in 100 ml plastic cups, which contained 5 g of each Chinese herb and 50 adults of *T. puterscentiae* and *A. ovatus*. Bean flour was used at five mass concentrations: 0%, 0.01%, 0.1%, 1%, and 10% (w/w). The previous concentrations of bean flour were well shaken individually with *C. pinnatifida* and *C. lachrymal-job* before the experiment start. The experimental cups were kept in an incubator at 25 °C, 85 ± 5% RH in the dark. The mortalities of *T. puterscentiae* and *A. ovatus* adults were estimated after 7, 14, 21 and 28 days. However, the population growth of

Table 1 Mortalities of *Tyrophagus puterscentiae* and *Aleuroglyphus ovatus* fed on Crataegus pinnatifida as influenced by different bean flour doses.

Bean flour concentrations (%)	*T. putrescentiae*				*A. ovatus*			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
0	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d
0.01	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d
0.1	15 c	24 c	32 c	40 c	6 c	13 c	19 c	26 c
1	49 b	58 b	70 b	81 b	28 b	35 b	43 b	52 b
10	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a

Means followed by different letters in the same column are significantly different.

Table 2 Mortalities of *Tyrophagus puterscentiae* and *Aleuroglyphus ovatus* fed on Coix lachrymal-jobi as influenced by different bean flour doses.

Bean flour concentrations (%)	*T. putrescentiae*				*A. ovatus*			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
0	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d
0.01	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d
0.1	26 c	37 c	49 c	61 c	15 c	24 c	32 c	51 c
1	54 b	69 b	80 b	92 b	29 b	40 b	54 b	69 b
10	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a

Means followed by different letters in the same column are significantly different.

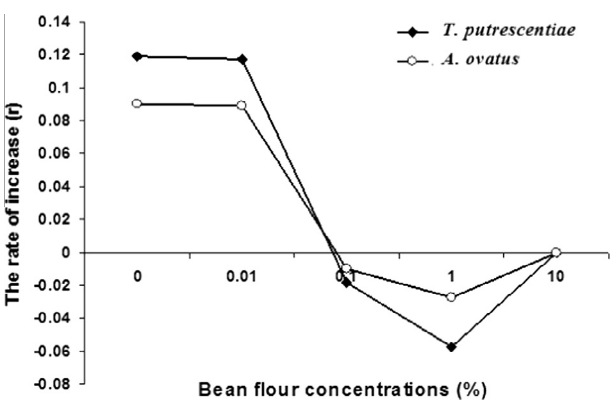

Figure 1 Rate of the increase (*r*) of *T. putrescentiae* and *A. ovatus* fed on Crataegus pinnatifida as influenced by bean flour concentrations.

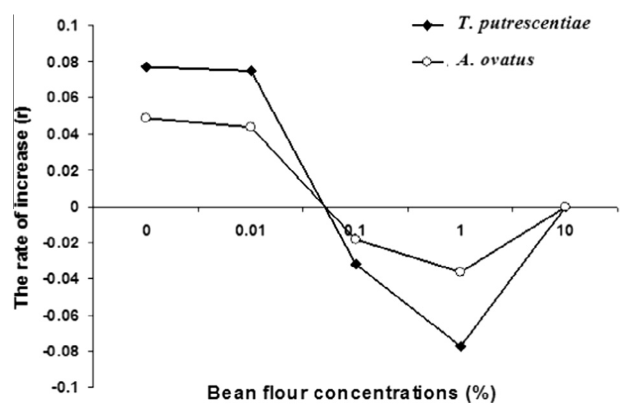

Figure 2 Rate of the increase (*r*) of *T. putrescentiae* and *A. ovatus* fed on Coix lachrymal-jobi as influenced by bean flour concentrations.

T. puterscentiae and *A. ovatus* was determined by a stereomicroscope after 21 days by extraction of living mites in Berlese–Tullgren funnels in a saturated solution of picric acid (Hubert et al., 2006). The population dynamics of *T. puterscentiae* and *A. ovatus,* which is expressed by the rate of increase (*r* value) was calculated using the differential densities-independent model ($Nt = N_0e^{rt}$) of McCallum (2000). The N_0 was the initial density of mites (= 50), Nt was the final density of mites, and *t* was the duration of the experiment (21 days).

2.4. Development of immature stages

The development of *T. putrescentiae* and *A. ovatus* immature stages on *C. pinnatifida* and *C. lachrymal-job* was determined at a constant temperature of 25 °C, under 85% RH and a photoperiod of 16:8 light: dark (L:D) as described by Kheradmand et al. (2007). The eggs of *T. putrescentiae* and *A. ovatus* were transferred after they were laid using a thin camel hair brush. After that, *T. putrescentiae* and *A. ovatus* were put individually on a filter paper containing *C. pinnatifida* or *C. lachrymal-job* on a Petri dish (9 cm diameter). The egg development was checked daily until maturity.

2.5. Statistical analysis

The data were statistically analyzed using the SAS software package, version 9.1 (SAS Institute Inc., Cary, NC, USA).

A one-way analysis of variance (ANOVA) was applied to determine and evaluate the effect of bean flour on mortality and population growth of storage mites at $P < 0.05$. The significant differences between the studied treatments were determined following the Tukey's honestly significant difference (HSD) test.

3. Results

3.1. Mortality of storage mites

The influence of bean flour on *T. puterscentiae* and *A. ovatus* mortalities fed on *C. pinnatifida* and *C. lachrymal-job* is presented in Tables 1 and 2. Addition of bean flour as an antifeedant led to significant increases in mortalities of *T. puterscentiae* and *A. ovatus* on *C. pinnatifida* and *C. lachrymal-job* as compared with the control treatment (zero % bean flour). The increase of storage periods of *T. puterscentiae* and *A. ovatus* with bean flour caused marked increases in *T. puterscentiae* and *A. ovatus* mortalities on *C. pinnatifida* and *C. lachrymal-job*. The toxic effects of bean flour concentrations were more obvious on *T. puterscentiae* than those on *A. ovatus*. This indicated that *T. puterscentiae* as compared with *A. ovatus* is more sensitive for bean flour. The controlling effect of bean flour for *T. putrescentiae* and *A. ovatus* on *C. pinnatifida* was lower than that on *C. lachrymal-job*. There were no mortalities noticed for *T. putrescentiae* and *A. ovatus* after all storage periods (7–28 days) when the bean flour was added at a concentration of 0.01% to *C. pinnatifida* and *C. lachrymal-job*. Addition of 0.1% bean flour was responsible for 40% and 26% mortalities of *T. putrescentiae* and *A. ovatus* on *C. pinnatifida* after 28 days. Moreover, the mortalities of *T. putrescentiae* and *A. ovatus* reached 61% and 51% when they fed on *C. lachrymal-job* under the previous concentration of bean flour (0.1%). After 28 days, the use of 1% bean flour caused 81% and 92% mortalities of *T. putrescentiae* and *A. ovatus* on *C. pinnatifida*. Furthermore, mortalities of *T. putrescentiae* and *A. ovatus* were 52% and 69% in the case of *C. lachrymal-job*. All individuals of *T. putrescentiae* and *A. ovatus* died due to the application of 10% of bean flour after all studied periods (7–28 days) on *C. pinnatifida* and on *C. lachrymal-job*.

3.2. Population dynamics of storage mites

The rate of increase (*r*) of *T. putrescentiae* and *A. ovatus* due to the addition of bean flour on *C. pinnatifida* and on *C. lachrymal-job* after 21 days is listed in Figs. 1 and 2. When the bean flour was added at a level of 0.01%, r values of *T. putrescentiae* and *A. ovatus* reared on *C. pinnatifida* and on *C. lachrymal-job* markedly declined with the increase of bean flour concentrations from 0.01% to 10%. At 0% concentration of bean flour, r values of *T. putrescentiae* and *A. ovatus* were 0.119 and 0.09 on *C. pinnatifida* and were 0.077 and 0.049 on *C. lachrymal-job*. Mixing of *C. pinnatifida* and *C. lachrymal-job* with 0.01%, 0.1%, 1% and 10% concentrations of bean flour was responsible for significant decreases in *r* values of *T. putrescentiae* and *A. ovatu*. The r values of *T. putrescentiae* and *A. ovatus* decreased to 0.117 and 0.089 on *C. pinnatifida* and to 0.075 and 0.044 on *C. lachrymal-job*. Conversely, negative *r* values were noticed at 0.1% and 1% of bean flour. The increase of bean flour concentrations from 0.1% to 1% caused marked diminishes in *r* values of *T. putrescentiae* and *A. ovatus* from −0.018 and −0.01 to −0.057 and −0.027 on *C. pinnatifida* and from −0.032 and −0.018 to −0.077 and −0.036 on *C. lachrymal-job*. On the other hand, *r* values of *T. putrescentiae* and *A. ovatus* on *C. pinnatifida* and on *C. lachrymal-job* were zero after their treating with 10% of bean flour.

4. Discussion

In the current investigation, we showed that the use of bean flour as an antifeedant was efficient in controlling *T. putrescentiae* and *A. ovatus* fed on *C. pinnatifida* and on *C. lachrymal-job*. Moreover, the results indicated the strong inhibitory effect of bean flour on the growth of *T. putrescentiae* and *A. ovatus*, which expressed with the rate of increase (*r* value). Hou and Fields (2003) revealed that the addition of 0.1% pea flour concentration on barley grains reduced the numbers of *Sitophilus oryzae* and *Tribolium castaneum* adults by 93% and 66%, respectively. Hubert et al. (2006) evaluated the effect of bean flour (*P. vulgaris*) on *T. putrescentiae* and *A. ovatus* and they found that bean flour had a more negative effect on *T. putrescentiae* than on *A. ovatus*. They also showed that the population growth (r value) was decreased to 50% in comparison to the control due to the use of 0.02% and 4.87% of bean flour concentrations on *T. putrescentiae* and *A. ovatus*, respectively. On the other hand, the concentration of 5% bean flour in diets kept populations of *A. siro* and *T. putrescentiae* at the initial level. The toxic effect of bean flour on *T. putrescentiae* and *A. ovatus* has not been discovered yet. However, Hubert et al. (2006) and Hubert et al. (2007) mentioned that unknown proteins, which are presented in the bean flour might be responsible for the inhibition of the digestion process in storage mites.

Our results showed that the decrease of *T. putrescentiae* and *A. ovatus* densities differed. These results are in agreement with the findings of Huber et al. (2006), Hubert et al. (2007) and

Table 3 Mean developmental time (days) of immature stages of reared on *Crataegus pinnatifida* or *Coix lachrymal-jobi* at 25 °C, 85% RH and 16:8 (L:D).

Stage	*T. putrescentiae*		*A. ovatus*	
	Crataegus pinnatifida	*Coix lachrymal-jobi*	*Crataegus pinnatifida*	*Coix lachrymal-jobi*
Egg	2.81 b	3.44 b	4.05 b	4.53 b
Larva	2.68 c	3.04 d	4.28 a	4.97 a
Protonymph	3.13 a	3.59 a	3.99 b	4.26 c
Tritonymph	2.79 b	3.22 c	3.61 c	3.88 d
Total	11.41	13.29	15.93	17.64

Means followed by different letters in the same column are significantly different. RH is relative humidity, while L:D is light to dark ratio.

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Hubert and Pekár (2009) who observed that the addition of bean flour was effective only against some stored product mites. They also mentioned that the toxic effect of bean flour on *T. putrescentiae* was higher that that on *A. ovatus*. This can be explained by the higher development (Table 3) of *T. putrescentiae* and *A. ovatus* on *C. pinnatifida* than on *C. lachrymal-job*. Therefore, it is very important to record that the toxic influence of bean flour as an antifeedant on *T. putrescentiae* and *A. ovatus* is highly related to the type of stored foods.

In conclusion, our results confirmed the importance of bean flour in the control of *T. putrescentiae* and *A. ovatus* fed on *C. pinnatifida* and *C. lachrymal-job*. The use of bean flour as a control strategy seems to be efficient and safe for preventing the infection of dried Chinese herbs with *T. putrescentiae* and *A. ovatus*. Furthermore, bean flour is one of human foods and has no negative effects on their health as compared with chemical pesticides. So, this study suggests that bean flour can be recommended as a good and eco-friendly antifeedant for storage mites in stores of Chinese herbs under the natural conditions. Further experiments are needed to be conducted in the future to examine the efficiency of bean flour in governing of stored product mites at different temperatures and humidities under natural conditions.

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