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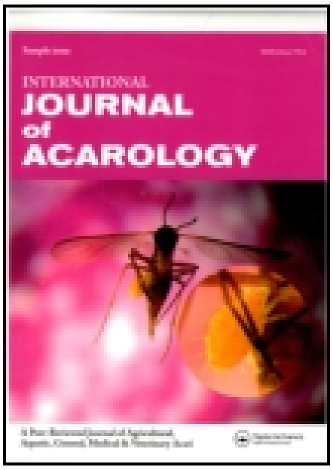

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Efficacy of pea flour as an antifeedant against two stored-food mites (Acari: Acaridae) fed on dried medicinal Chinese herbs

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Pea flour (*Pisum sativum* L.) is toxic to some stored-product pests. Botanical pesticides that contained efficient natural compounds have highlighted to be used for the control of storage mites. In the current investigation, we evaluated the effect of pea flour as an antifeedant on two stored-product mites, namely *Tyrophagus putrescentiae* (Schrank, 1781) and *Aleuroglyphus ovatus* (Troupeau, 1878) fed on two medicinal Chinese herbs: *Crataegus pinnatifida* Bge. var. major and *Coix lachryma-jobi* L. var. *ma-yuen*. The experiment was carried out from March to May 2012 under the optimal growth conditions of storage mites at constant temperature (25°C) and 85 ± 5% relative humidity (RH) in the dark. Pea flour was used at five concentrations (0, 0.01, 0.1, 1 and 10%). The values of LC_{50}, LC_{90} and mortality of *T. putrescentiae* and *A. ovatus* were recorded at four periods (7, 14, 21 and 28 days), while their population dynamics were determined only after 21 days. The use of pea flour as an antifeedant was very efficient for the control of *T. putrescentiae* and *A. ovatus* adults. The controlling efficiency of pea flour improved due to the increase of its applied dose. The LC_{50} and LC_{90} estimates showed that pea flour was more toxic for *T. putrescentiae* than for *A. ovatus*. The concentration of 1% pea flour was responsible for more than 65% and 88% mortality of *A. ovatus* on *C. pinnatifida* and *C. lachryma-jobi* after 28 days, respectively. However, this concentration was sufficient to kill all individuals (100% mortality) of *T. putrescentiae* as the concentration of 10% pea flour. The addition of pea flour caused considerable decreases in the rate of the increase (*r*-value) of *T. putrescentiae* and *A. ovatus*. The highest *r*-values of *T. putrescentiae* and *A. ovatus* were recorded on the control treatment (0% pea flour), whereas the lowest values were observed at 1% and 10% concentrations of pea flour. The 1% concentration of pea flour is highly suggested to be used as a good and economical dose to control both of *T. putrescentiae* and *A. ovatus* on the studied Chinese herbs.

Keywords: pea flour; storage mites; Chinese herbs; mortality; population dynamic

Introduction

Mites are important pests and considered as a major problem for stored food products and have a worldwide distribution, especially in these countries which characterized with a humid climate (Athanassiou et al. 2005). Storage mites such as *Tyrophagus putrescentiae* (Schrank, 1781) and *Aleuroglyphus ovatus* (Troupeau, 1878) can be found in a wide variety of stored products including dried fruits, vegetables, grains, seeds and medicinal Chinese herbs (Hughes 1976).

Chinese pearl barley (*Coix lacryma-jobi*) is an annual grass crop that is widely cultivated in oriental countries such as China, Taiwan, Japan, Thailand and Korea, and has long been used as a traditional medicine and a nourishing food (Bhandari et al. 2012). It was recorded that *C. lacryma-jobi* seeds succeeded in curing many diseases, such as warts, chapped skin, rheumatism and neuralgia, and can be used as an anti-inflammatory and an anthelmintic agent (Hsu et al. 2003). Moreover, *C. lacryma-jobi* has important roles in the suppression of colon carcinogenesis (Shih et al. 2004) and in reducing the blood lipids (Yu et al. 2011). Hawthorn is the common name of *Crataegus pinnatifida* and is generally distributed in China as herbal medicine and food materials (Zhao and Tian 1996). Many studies showed that *C. pinnatifida* can treat various cardiovascular diseases, including myocardial weakness, hypertension and arteriosclerosis (Chang et al. 2002), and have anti-inflammatory properties and high capacity to inhibit the oxidation of low-density lipoprotein in cells (Chu et al. 2003; Kao et al. 2005).

Mite infestation is associated with negative effects on the quality and safety of stored products through the accumulation of dead mites, faeces, exuviae, eggs and bits of food (Peace 1983), and fungi mycotoxins (Hubert et al. 2003). In addition, these mites are responsible for allergic diseases among farmers and food industry workers (Hughes 1976) and may cause acute enteritis, pulmonary acariasis, urinary acariasis, diarrhoea and systemic anaphylaxis (Hughes 1976; Matsumoto and Satoh 2004). Chemical control of storage mites, which depends on acaricides application, especially organophosphates can lead to serious problems and become prohibited due to their hazardous effects on human health (Sanchez-Ramos and Castanera 2001; Collins 2006). Thus, it is very important to find and test new methods characterized by high ability in the control of storage mites without any toxic influences on humans.

Plant-derived products such as botanical pesticides can be used as potential sources for storage pests control through oviposition deterrent and growth disruptor

(Holloway 1986; Schmutterer 1990; Isman 2000; Koul et al. 2008). It was reported by Lee et al. (2004) that botanical pesticides have few or no harmful effects on the environment and human. The application of pea flour as an antifeedant caused high reductions in populations, reproductions and survival rates of many stored-product pests such as *Sitophilus oryzae* L., *Sitophilus zeamais* Motschulsky L., *Sitophilus granarius* L., *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* F. (Holloway 1986; Bodnaryk et al. 1999; Fields et al. 2001; Hou and Fields 2003; Fields 2006). Although many studies evaluated the effect of legume flour such as pea flour (*Pisum sativum* L.) or bean flour (*Phaseolus vulgaris* L.) on stored-product insects, few reports are available about the control of storage mites by bean flour (Hubert et al. 2006, 2007; Hubert and Pekár 2009). To the best of our knowledge, there is no investigation has been carried out to estimate the effectiveness of pea flour as an antifeedant against storage mites. Therefore, the objective of this study was to examine the effect of pea flour at different concentrations on the mortality and population dynamics of two storage mites (*T. putrescentiae* and *A. ovatus*) reared on two dried medicinal Chinese herbs (*C. pinnatifida* Bge. var. major and *Coix lachryma-jobi* L. var. *ma-yuen*).

Materials and methods

Field pea and Chinese herbs

Seeds of field yellow pea (*Pisum sativum* L.) were used as an antifeedant in the current study and were obtained from the College of Horticulture, while the Chinese herbs (*C. pinnatifida* and on *C. lachryma-jobi*) were collected from the College of Plant Science and Technology at Huazhong Agricultural University, Wuhan City, Hubei Province, China. Pea seeds and Chinese herbs were dried at 60°C for 6 h as described in the study of Hubert and Pekár (2009). Afterward, pea seeds were ground to powder by a high-speed universal disintegrator (FW80, Hebei, China), passed through a 0.053 mm sieve and stored in polyethylene bags for the future using. The Chinese herbs were crushed into small pieces (<2 mm) by an electric grinding mill (ACME, AM-128, Hubei, China).

Storage mites

Two species of stored-product mites were used for the experiment, *T. putrescentiae* and *A. ovatus*. These mites for our research were collected from old infected wheat flour samples at the College of Plant Science and Technology, Huazhong Agricultural University, Wuhan City, Hubei Province, China. The pervious mites were mass-reared in glass flasks (1000 ml) and were covered by muslin and placed into a Mould Cultivation Cabinet (MJX-70BIII, Cangzhou, Hebei, China) at 85 ± 5% RH and 25°C under darkness condition (Hubert et al. 2006). The rearing diet consists of 45 g oat flakes, 45 g wheat flour and 10 g yeast (Hubert et al. 2006). After getting a high mass production of mites, they were transferred individually by a camel hair brush (Hubert et al. 2006) to new rearing flasks (250 ml) had 50 g Chinese herbs for one week to conduct the acclimation process at 85 ± 5% RH and 25°C in the dark before the experiment establishment.

Experimental design

The experiment was factorial with three factors (herbs, storage mites and pea flour concentrations) and was set up in a randomized complete block design with 10 replicates for each mite, Chinese herb and pea flour concentration. The experiment was carried out in 100 ml plastic cups, contained 5 g of each Chinese herb and 50 adults of *T. putrescentiae* or *A. ovatus*. Pea flour was added to Chinese herbs in five mass concentrations: 0, 0.01, 0.1, 1 and 10% (wt/wt) as suggested by Fields et al. (2001) and Fields (2006), using Ohaus microbalance with 0.01 g accuracy (Ohaus Corp Pine Brooke, NJ, USA). The previous concentrations of the pea flour were manually shaken (4–5 times) with the Chinese herbs to get good mixtures of them. The experimental cups were placed into a Mould Cultivation Cabinet (MJX-70BIII, China) at 25°C and 85 ± 5% RH in the dark (Hubert et al. 2006; Hubert et al., 2007). The mortality of *T. putrescentiae and A. ovatus* adults was estimated after 7, 14, 21 and 28 days. However, the population dynamics of *T. putrescentiae and A. ovatus* were determined after 21 days by extraction of living mites in Berlese–Tullgren funnels (Hubert et al. 2006). The *T. putrescentiae and A. ovatus* were collected individually in a saturated solution of picric acid (Hubert et al. 2006, 2007) and accounted under a stereo microscope (MZS1865C, Guangxi, China). The population growth or the rate of the increase (r) of *T. putrescentiae* and *A. ovatus* was calculated using the differential density-independent model ($N_t = N_0 e^{rt}$) of McCallum (2000). This formula can be solved as $rt = \ln(N_t/N_0)$. The N_0 was the initial density of mites (50), N_t was the final density of mites, and t was the experiment duration (21 days). The yeast, muslin, camel hair brush, polyethylene bags, glass flacks, plastic cups and picric acid were obtained from Wuhan Bolan Scientific Chemicals and Equipment Co., Ltd., Hubei, China.

Data analysis

The data was statistically analysed using the SAS software package, version 9.1 (SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) was conducted to evaluate the effect of pea flour on the mortality and population growth of storage mites. The significant differences between the studied treatments were determined by the Tukey's test at $P < 0.05$. The Probit analysis (Finney 1971) using program Polo-PC (LeOra-Software 1987) was applied to calculate the LC_{50} and LC_{90} values for *T. putrescentiae* and *A. ovatus* as affected by different pea flour concentrations.

Results

Toxicity of pea flour

Data in Table 1 showed the toxic doses of pea flour which killed 50% and 90% (LD_{50} and LD_{90}) of *T. putrescentiae and A. ovatus* which reared on *C. pinnatifida* and *C. lachryma-jobi*. The LD_{50} and LD_{90} values of pea flour on *A. ovatus* were higher than those on *T. putrescentiae*. Furthermore, these values were greater on *C. pinnatifida* than on *C. lachryma-jobi*. In the case of *T. putrescentiae* infection, the LD_{50} and LD_{90} ranged from 0.53% to 0.07% and from 5.88% to 0.77% on *C. pinnatifida*, while they varied from 0.33% to 0.02% and from 5.62% to 0.30% on *C. lachryma-jobi*, respectively. When *A. ovatus* attacked *C. pinnatifida* and *C. lachryma-jobi*, the LD_{50} changed from 1.20% to 0.37% and from 0.63% to 0.05%, and the LD_{90} differed from 8.37% to 4.90% and from 6.95% to 0.44%, respectively.

Mortality of storage mites

Addition of pea flour as an antifeedant led to significant increases in the mortality of *T. putrescentiae and A. ovatus* on *C. pinnatifida* and *C. lachryma-jobi* (Figures 1A, B; 2A, B). Mortality percentages of *T. putrescentiae and A. ovatus* enlarged due to the increase of storage periods between the pea flour and *C. pinnatifida* and *C. lachryma-jobi*. There was no mortality noticed at all experimental durations (7, 14, 21 and 28 days) for *T. putrescentiae* and *A. ovatus* at 0% concentration of pea flour, so the corrected mortality did not calculate. When the pea flour was added in the concentration of 0.01% to *T. putrescentiae* on *C. pinnatifida*, there was no mortality recorded at 7 and 14 days. On the other hand, the mortality of *T. putrescentiae* increased to 13% and 19% at 21 and 28 days, respectively. In the case of *C. lachryma-jobi*, the mortality of *T. putrescentiae* varied from 5% at 7 days to 33% at 28 days. The use of 0.01% concentration of pea flour was not effective to control the *A. ovatus* on *C. pinnatifida* at 7, 14, 21 and 28 days. The mortality of *A. ovatus* on *C. lachryma-jobi* was zero at 7 and 14 days, but it enhanced to 9% and 20% after 21 and 28 days, respectively. Application of 0.1% concentration pea flour decreased the numbers of *T. putrescentiae* and *A. ovatus* by 51% and 29% on *C. pinnatifida*, and by 75% and 66% on *C. lachryma-jobi* after 28 days. At 1% concentration of pea flour, the mortality of *T. putrescentiae* was 87% on *C. pinnatifida* and 100% on *C. lachryma-jobi* after 21 days. Additionally, all individuals of *T. putrescentiae* were dead after 28 days. When 1% concentration of pea flour was used to control *A. ovatus*, the mortality was 66% and 88% after 28 days on *C. pinnatifida*. The mortality of *T. putrescentiae* and *A. ovatus* was 100% as a result of 10% concentration of pea flour addition at 7, 14, 21 and 28 days on *C. pinnatifida* and *C. lachryma-jobi*.

Population dynamics of storage mites

The rate of the increase (r) of *T. putrescentiae* and *A. ovatus* as influenced by different pea flour concentrations on *C. pinnatifida* and *C. lachryma-jobi* after 21 days is illustrated in Figure 3A and B. The values of r of *T. putrescentiae* and *A. ovatus*, which reared on *C. pinnatifida* and *C. lachryma-jobi*, declined markedly due to the increase of pea flour concentrations. At 0% concentration of pea flour, r-values of *T. putrescentiae* and *A. ovatus* were 0.115 and 0.092 on *C. pinnatifida* and 0.084 and 0.021 on *C. lachryma-jobi*, respectively. The use of 0.01%, 0.1%, 1% and 10% concentrations of pea flour caused significant decreases in r-values of *T. putrescentiae* on *C. pinnatifida* (–0.002, –0.027, –0.097 and 0, respectively) and on *C. lachryma-jobi* (–0.106, –0.041, 0 and 0) at the end of population dynamic experiment (21 days). The r-value of *A. ovatus* was 0.016 due to the application of 0.01% concentration of pea flour on *C. pinnatifida* and then decreased to –0.014, –0.045 and 0 when pea flour was added at 0.1%, 1% and 10% concentrations, respectively. Also, r-value of

Table 1. Acaricidal activity of pea flour against *T. putrescentiae* and *A. ovatus*.

Mites	Chinese herbs	Time (day)	LD_{50} (%) (95% Cl)	LD_{90} (%) (95% Cl)	Slope ± SE
T. putrescentiae	*C. pinnatifida*	7	0.53 (0.26–1.02)	5.88 (2.15–25.3)	1.25 ± 0.44
		14	0.28 (0.14–0.48)	3.09 (1.78–5.89)	1.22 ± 0.38
		21	0.13 (0.07–0.20)	1.60 (1.04–2.50)	1.16 ± 0.32
		28	0.07 (0.01–0.22)	0.77 (0.26–2.13)	1.25 ± 0.55
	C. lachryma-jobi	7	0.33 (0.09–0.87)	5.62 (2.79–13.6)	1.02 ± 0.42
		14	0.13 (0.09–0.18)	2.15 (1.62–2.89)	1.05 ± 0.23
		21	0.06 (0.02–0.13)	0.61 (0.28–1.23)	1.25 ± 0.46
		28	0.02 (0.01–0.04)	0.30 (0.21–0.42)	1.17 ± 0.30
A. ovatus	*C. pinnatifida*	7	1.20 (0.53–2.80)	8.37 (2.26–85.1)	1.68 ± 0.77
		14	0.72 (0.28–1.77)	7.20 (2.85–27.1)	1.29 ± 0.53
		21	0.47 (0.18–1.04)	5.76 (2.48–17.9)	1.17 ± 0.45
		28	0.37 (0.14–0.83)	4.90 (2.16–14.5)	1.15 ± 0.43
	C. lachryma-jobi	7	0.63 (0.13–2.33)	6.95 (2.96–22.5)	1.14 ± 0.54
		14	0.34 (0.08–1.01)	5.31 (1.76–27.7)	1.08 ± 0.47
		21	0.16 (0.11–0.22)	2.29 (1.73–3.09)	1.10 ± 0.24
		28	0.05 (0.03–0.06)	0.44 (0.36–0.55)	1.30 ± 0.26

Note: SE, standard error; Cl, Confidence limit; LD_{50} and LD_{90} are lethal doses for 50% and 90% mortality of mites.

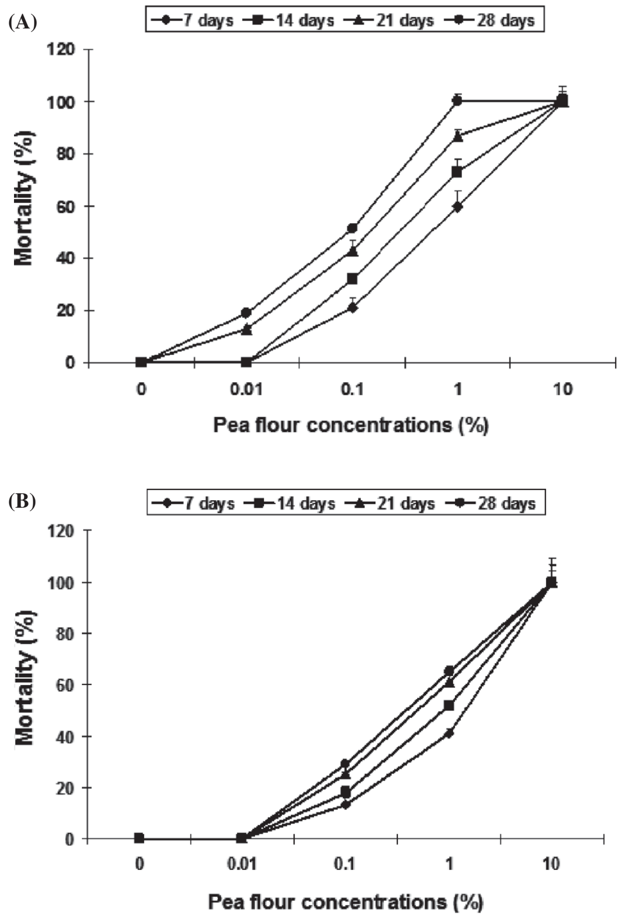

Figure 1. Mortality of *T. putrescentiae* (A) *and A. ovatus* (B) fed on *C. pinnatifida* as influenced by pea flour. The small bars express the values of standard errors.

A. ovatus declined to –0.005, –0.026, –0.057 and 0 after the combination of *C. lachryma-jobi* with 0.01%, 0.1%, 1% and 10% concentrations of pea flour, respectively.

Discussion

Our results indicated the strong efficient influence of pea flour on the mortality and population growth of storage mites. Similarly, Hou and Fields (2003) showed that the addition of 0.1% pea flour concentration on barley grains increased the mortality of two stored-product pests (*Sitophilus oryzae* and *Tribolium castaneum*) by 93% and 66%, respectively. It was found by Fields (2006) that the use of fresh protein-rich pea flour in a concentration of 0.1% can be responsible for 90% mortality of *S. oryzae* after two weeks when it held on wheat kernels. Hubert et al. (2006) indicated that the addition of bean flour in concentrations of 0.04% and 4.87% increased the mortality of *T. putrescentiae* and *A. ovatus* on a mixture of oat flakes, wheat germ, lyophilized yeast and dried Daphnia (44, 44, 10 and 2 g, respectively) to 50% in comparison to the control (0% bean flour) after 21 days. Moreover, they mentioned that the concentration of 5% bean flour led to 100% mortality of *T. putrescentiae*. In another study by Hubert et al.

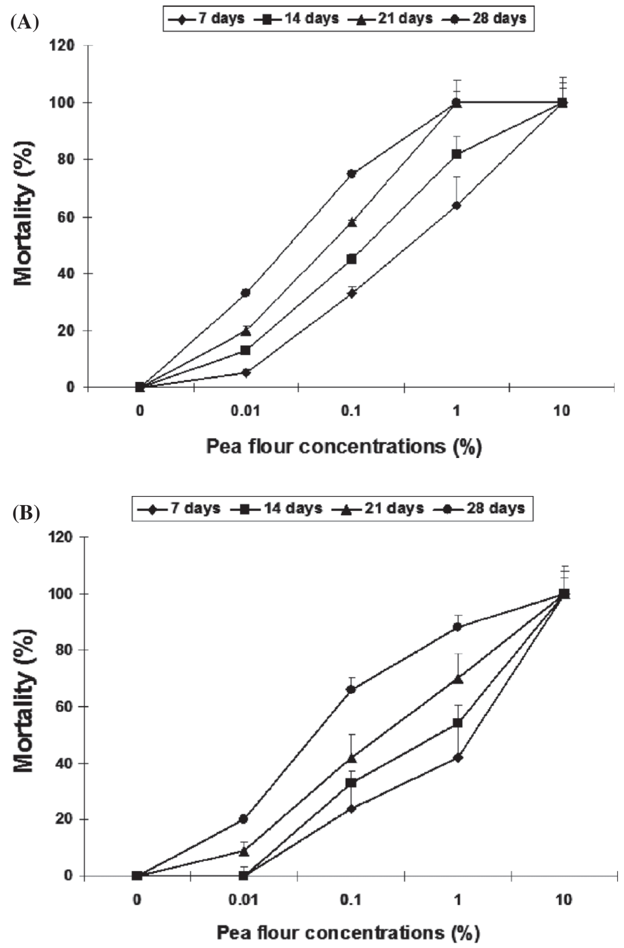

Figure 2. Mortality of *T. putrescentiae* (A) and *A. ovatus* (B) reared on *C. lachryma-jobi* as influenced by pea flour. The small bars express the values of standard errors.

(2007), the use of bean flour (<concentration of 1.5%) can be enough to kill all *T. putrescentiae* after 21 days on a mixture of a mixture of oat fakes and wheat germ (1:1). This can be illustrated by the presence of a wide range of natural compounds such as allelochemicals in legume flour which have toxic and deterrent effects on stored-product pests (Bell 1978). The mortality of stored food pests increased after the addition of whole-pea flour due to the presence of starch (Pretheep-Kumar et al. 2004). However, Bodnaryk et al. (1999) showed that the presence of protein could be responsible for the repellent effect of pea flour against these pests.

The increase of pea flour concentration caused significant increases in the mortality of storage mites. These results are in agreement with the findings of other researchers (Hubert et al. 2006, 2007; Hubert and Pekár 2009). It is very important to record that the influence of pea flour as an antifeedant on the *T. putrescentiae* and *A. ovatus* is highly related to the mite species and the type of stored food. It was recorded in different investigations carried out by Hubert et al. (2006), Hubert et al. (2007) and Hubert and Pekár (2009) that bean flour was more toxic on *T. putrescentiae* than on *A. ovatus*. The higher controlling

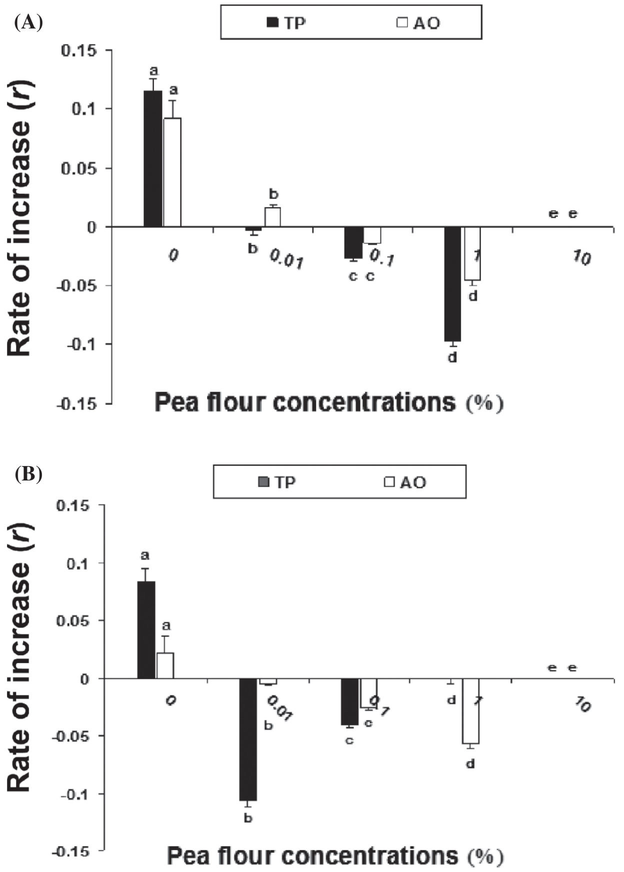

Figure 3. Rate of increase (r) of *T. putrescentiae* (TP) *and A. ovatus* (AO) held on *C. pinnatifida* (A) and on *C. lachryma-jobi* (B) as influenced by pea flour. The small bars express the values of standard errors. Different letters on bars are significantly different at $p < 0.05$ due to the Tukey's test.

effect of pea flour for *T. putrescentiae* and *A. ovatus* on *C. pinnatifida* can be explained by the larger development, growth and reproduction of *T. putrescentiae* and *A. ovatus* on *C. pinnatifida* than those on *C. lachryma-jobi* (data not shown).

It is clear from our data that the increase of mortality of *T. putrescentiae* and *A. ovatus* after the use of pea flour was responsible for marked decreases in their population growth values, which is explained by the rate of the increase (r-value). These findings are in harmony with the results of Hubert and Pekár (2009), who demonstrated that r-values of *T. putrescentiae* and *A. ovatus* decreased when pea flour concentrations increased.

This is the first report providing important information for the producers of medicinal Chinese herbs about the influence of pea flour as an antifeedant against storage mites. Further studies should be conducted to evaluate the combination effect of pea flour and predatory mites on storage mites on dried Chinese herbs at different temperature and relative humidity.

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