

Original Research Article

Insecticidal Activity of Essential Oil of *Cinnamomum cassia* and its Main Constituent, *trans*-Cinnamaldehyde, against the Booklice, *Liposcelis bostrychophila*

Xin Chao Liu¹, Jun Cheng², Na Na Zhao¹ and Zhi Long Liu^{1*}

¹Department of Entomology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, ²Key Laboratory of Urban Agriculture (North), Ministry of Agriculture, Beijing University of Agriculture, Haidian District, Beijing 102206, China

*For correspondence: **Email:** zhilongliu@cau.edu.cn; **Tel:** +86-10-62732800; **Fax:** +86-10-62732800

Received: 3 July 2014

Revised accepted: 9 September 2014

Abstract

Purpose: To investigate the insecticidal activity of the essential oil of *Cinnamomum cassia* and its main constituent compound, *trans*-cinnamaldehyde, against the booklice, *Liposcelis bostrychophila*.

Methods: Steam distillation of *C. cassia* twigs was carried out using a Clavenger apparatus in order to obtain the volatile oils. Gas chromatography/mass spectrometric (GC/MS) analyses (HP-5MS column) of the essential oil were performed and its contact (using impregnated filter paper method) and fumigant toxicity (sealed space) determined. The bioactive constituent compound, *trans*-cinnamaldehyde was isolated and identified from the oil based on bioactivity-directed fractionation.

Results: A total of 35 components, accounting for 97.44 % of the essential oil of *C. cassia*, were identified. The principal compounds in the essential oil were *trans*-cinnamaldehyde (49.33 %), acetophenone (6.94 %), *trans*-cinnamic acid (5.45 %) and *cis*-cinnamaldehyde (4.44 %) followed by *o*-methoxycinnamaldehyde (3.48 %), coumarin (3.42 %) and (*E*)-cinnamyl alcohol (3.21 %). The essential oil displayed contact toxicity against adult *L. bostrychophila* with a median lethal concentration (LC₅₀) of 55.68 µg/cm² as well as fumigant toxicity (LC₅₀, 1.33 mg/l air). *Trans*-cinnamaldehyde exhibited strong contact and fumigant toxicity with LC₅₀ of 43.40 µg/cm² and 1.29 mg/l air, respectively.

Conclusion: The findings suggest that the essential oil of *C. cassia* and its constituent compound, *trans*-cinnamaldehyde, possess potentials for development into natural fumigants/insecticides for the control of booklice.

Keywords: *Liposcelis bostrychophila*, *Cinnamomum cassia*, Contact toxicity, Fumigant, *trans*-Cinnamaldehyde, Essential oil

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The booklice (*Liposcelis bostrychophila* Badonnel; Psocoptera: Liposcelidae) are frequently found in stored-product grains, often in extremely high numbers, in amylaceous products [1]. Infestations of stored product insects could be controlled by fumigation or insecticidal treatment of commodities and surfaces, which

has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to the users. These problems have highlighted the need to develop new types of selective insect-control alternatives. Investigations in several countries confirm that some essential oils not only repel insects, but

possess contact and fumigant toxicity against stored product pests as well as exhibited feeding inhibition or harmful effects on the reproductive system of insects. Essential oils or their constituents may provide an alternative to currently used fumigants/insecticides to control stored-food insects [2].

Cinnamomum cassia Presl (Family: Lauraceae) evidently originates in southern China, but now widely cultivated in tropical or subtropical areas, e.g., India, Indonesia, Laos, Malaysia, Thailand and Vietnam [3]. The twigs and bark of this species are widely consumed in Asia as a spice and commonly used as traditional Chinese medicine for treating dyspepsia, gastritis, blood circulation disturbances, and inflammatory diseases [4].

The chemical composition of the essential oil of *C. cassia* has been widely studied [5-8]. This essential oil possesses contact and fumigant toxicity against several stored product insects and house dust mites [9-13]. Moreover, the essential oil of *C. cassia* also exhibits strong repellency and larvicidal activity against several mosquitoes [14]. However, no information on insecticidal activity of the essential oil of *C. cassia* twigs and its main constituent compounds against booklice is available in the literature, to the best of our knowledge.

The aim of this study is to evaluate contact and fumigant toxicity of the essential oil against *L. bostrychophila* and the isolation of the active constituent compound.

EXPERIMENTAL

Plant material and essential oil

The dried twigs of *C. cassia* (3 kg) were purchased from Guangming Chinese Medicinal Herb Decoction Company (Anguo City, Hebei 071200, China). The herb was identified by Dr Liu, QR (College of Life Sciences, Beijing Normal University, Beijing 100875, China) and a voucher specimen was deposited in the museum of Department of Entomology, China Agricultural University (no. ENTCAU-Lauraceae-10012). The herb was first ground to powder using a grinding mill (Retsch Muhle, Germany) and subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h, and then extracted with *n*-hexane. Anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in airtight containers in a refrigerator at 4°C pending subsequent experiments.

Analysis of the essential oil

Capillary gas chromatography was performed using Hewlett–Packard 5890 gas chromatograph equipped with a flame ionization detector; fused silica capillary column HP-5 (5 % diphenyl and 95 % dimethylpolysiloxane, 30 m × 0.25 mm, 0.25 µm film thickness); at a flow rate of 1 mL min⁻¹; and programmed temperature from 60 to 280 °C (at a rate of 2 °C min⁻¹); injector temperature 270 °C and detector temperature 300 °C.

The components of the essential oil were separated and identified by gas chromatography–mass spectrometry (GC–MS) using Agilent 6890N gas chromatography coupled to Agilent 5973N mass selective detector. The system was equipped with a flame ionization detector and capillary column with HP-5MS (30 m × 0.25 mm × 0.25 µm). GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and ramped at 10 °C min⁻¹ to 180 °C where it was held for 1 min, and then ramped at 20 °C min⁻¹ to 280 °C and held there for 15 min. The injector temperature was maintained at 270 °C. The samples (1 µl) were injected neat, with a split ratio of 1: 10. The carrier gas was helium at a flow rate of 1.0 ml min⁻¹. Spectra were obtained over the scan range 20 to 550 m/z at 2 scans s⁻¹.

Most constituents were identified by gas chromatography by comparison of their retention indices with those published in the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C8–C24) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [15]. Relative percentages of the oil components were calculated based on GC peak areas without using correction factors.

Insects

Booklouse, *L. bostrychophila*, was obtained from laboratory cultures in the dark in incubators at 28 - 30 °C and 70 - 80 % relative humidity and reared on a 1: 1: 1 mixture, by mass, of milk powder, active yeast, and flour. All containers housing insects and the petri dishes used in experiments were made escape proof with a coating of polytetrafluoroethylene (Fluon, Blades Biological, UK). Adult insects used in all the experiments were about one week old.

Contact toxicity test

Range-finding studies were run to determine the appropriate testing concentrations of the essential oil of *C. cassia* and pure compound. The essential oil and compound were diluted in acetone. The filter paper with 3.5 cm in diameter (Whatman) was treated with 150 μ l of the solution. The treated filter paper after treated with solid glue (Glue Stick, Jong le Nara Co Ltd, Hong Kong) was placed in a Petri dish (3.5 cm in diameter) and 10 booklice were put on the filter paper. The plastic cover with holes was placed over it and all the Petri dish kept in incubators at 27 - 29 °C/70 - 80% RH for 24 h. Acetone was used as controls and pyrethrum extract was used as a positive control. Five concentrations and five replicates of each concentration were used in all treatments and controls. Mortality of insects was observed. Pyrethrum extract (25 % pyrethrin I and pyrethrin II) was purchased from Fluka Chemie.

Fumigant toxicity bioassay

Range-finding studies were run to determine the appropriate testing concentrations of the pure compound and *C. cassia* essential oil. A filter paper strip (3.5 cm x 1.5 cm) treated with 10 μ l of an appropriate concentration of test essential oil/compound in acetone. The impregnated filter paper was then placed in the bottom cover of glass bottle of 250 ml. The insects, 10 adults with undefined sex in a small glass bottle (8 ml), were exposed for 24 h and each concentration with five replicates. All the treatments were replicated five times. Acetone was used as controls and dichlorvos was used as a positive control. Mortality of insects was observed. Positive control, dichlorvos (99.9 %) was purchased from Aladdin-reagent Company (Shanghai).

Bioassay-directed fractionation

The crude essential oil of *C. cassia* (25 ml) was chromatographed on a silica gel (Merck 9385, 1,000 g) column (85 mm i.d., 850 mm length) by gradient elution with a mixture of solvents (n-hexane, n-hexane-ethyl acetate). Fractions of 500 ml were collected and concentrated at 40 °C, and similar fractions according to TLC profiles were combined to yield 15 fractions. Fractions (4-6) that possessed contact toxicity, with similar TLC profiles, were pooled and further purified by preparative silica gel column chromatography (PTLC) until to obtain the pure compound for determining structure as trans-cinnamaldehyde (0.6 g). The structure of the compound was elucidated based on high-resolution electron impact mass spectrometry

and nuclear magnetic resonance. ^1H and ^{13}C NMR spectra were recorded on Bruker ACF300 [300MHz (^1H)] and AMX500 [500MHz (^1H)] instruments using CDCl_3 as the solvent with TMS as internal standard. EIMS were determined on a ThermoQuest Trace 2000 mass spectrometer at 70 eV (probe).

Statistical analysis

Fumigant and contact toxicity data were subjected to Probit analysis using PriProbit Program V1.6.3 to determine LC_{50} values, respectively [16]. Samples for which the 95 % fiducial limits did not overlap were considered to be significantly different.

RESULTS

The chemical composition of the essential oil of *C. cassia* twigs was summarized in Table 1. A total of 35 components, accounting for 97.44 % of the essential oil of *C. cassia*, were identified. The principal compounds in the essential oil were trans-cinnamaldehyde (49.33 %), acetophenone (6.94 %), trans-cinnamic acid (5.45 %) and cis-cinnamaldehyde (4.44 %) followed by o-methoxycinnamaldehyde (3.48 %), coumarin (3.42 %) and (E)-cinnamyl alcohol (3.21 %).

trans-Cinnamaldehyde (Figure 1), Colorless oil. EI-MS m/z (%): 132 (75), 131 (100), 104 (27), 103 (55), 78 (26), 77 (36), 51 (21). $\text{C}_9\text{H}_8\text{O}$. ^1H -NMR (500 MHz, CDCl_3) δ (ppm): 9.67 (1H, d, J = 7.8 Hz, H-9), 7.53 (2H, d, J = 16.0 Hz, H-2, 6), 7.43 (1H, dd, J = 15.9 Hz, H-7), 7.38-7.42 (3H, m, H-3,4,6), 6.69 (1H, dd, J = 7.7 and 15.9 Hz, H-8). ^{13}C -NMR (125 MHz, CDCl_3) δ (ppm): 193.36 (C-9), 152.46 (C-7), 134.12 (C-1), 131.17 (C-8), 129.07 (C-3, C-5), 128.62 (C-4), 128.46 (C-2, C6). The ^1H and ^{13}C NMR data were in agreement with the reported data [17].

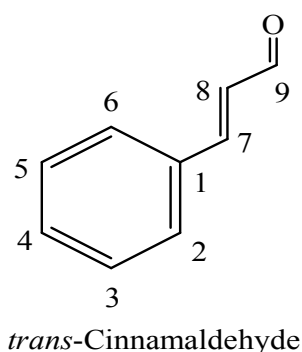


Figure 1: Structure of the main constituent isolated from *Cinnamomum cassia*

Table 1: Chemical constituents of the essential oil of *Cinnamomum cassia*

Peak no.	Compound	Retention index	%
1	α -Pinene	939	0.54
2	camphene	954	0.28
3	Benzaldehyde	961	2.76
4	β -Pinene	981	0.32
5	(+)-4-Carene	1002	0.26
6	β -Phellandrene	1026	0.61
7	Acetophenone	1066	6.94
8	β -Terpineol	1145	0.08
9	Benzenepropanal	1163	0.64
10	Borneol	1167	1.22
11	4-Terpineol	1175	0.21
12	α -Terpineol	1189	1.01
13	cis-Cinnamaldehyde	1219	4.44
14	Coumaran	1226	1.21
15	Hydrocinnamic alcohol	1236	3.11
16	trans-Cinnamaldehyde	1266	49.33
17	Bornyl acetate	1285	0.75
18	(E)-Cinnamyl alcohol	1315	3.21
19	α -Cubebene	1350	0.16
20	Citronellol acetate	1354	0.67
21	α -Ylangene	1372	0.53
22	Copaene	1374	0.11
23	Geraniol acetate	1381	0.23
24	β -Caryophyllene	1420	0.47
25	Coumarin	1432	3.42
26	trans-Cinnamic acid	1458	5.45
27	Chamigrene	1478	0.68
28	α -Curcumene	1484	0.89
29	α -Muurolene	1498	0.31
30	o-Methoxycinnamaldehyde	1502	3.48
31	β -Bisabolene	1506	0.92
32	δ -Cadinene	1523	0.16
33	(-)-Spathulenol	1578	0.47
34	Caryophyllene oxide	1581	2.33
35	τ -Muurolol	1642	0.37
	Total		97.57

Table 2: Insecticidal activity of the essential oil of *Cinnamomum cassia* and some of its isolated compounds against adult *Liposcelis bostrychophila*

Treatment	Contact toxicity			Fumigant toxicity		
	LC ₅₀ ($\mu\text{g}/\text{cm}^2$) (95%FL)	Slope \pm SE	Chi square (χ^2)	LC ₅₀ (mg/l air) (95%FL)	Slope \pm SE	Chi square (χ^2)
C. cassia	55.68	5.82 \pm 0.48	11.56	1.33	4.20 \pm 0.43	15.65
Mean range	(49.56-60.39)			(1.20-1.46)		
trans-Cinnamaldehyde	43.40	5.58 \pm 0.46	9.32	1.29	5.58 \pm 0.56	14.56
Mean range	(38.66-47.06)			(1.20-1.42)		
Pyrethrum extract	18.99	7.64 \pm 1.05	7.78	-	-	-
Mean range	(17.56-20.06)					
Dichlorvos	-	-		1.35 \times 10 ⁻³	6.87 \pm 0.77	12.13
Mean range				(1.25-1.47) \times 10 ⁻³		

LC₅₀ value of 43.40 $\mu\text{g}/\text{cm}^2$ while the essential oil of *C. cassia* twigs had an LC₅₀ value of 55.68 $\mu\text{g}/\text{cm}^2$ (Table 2). The crude essential oil of *C. cassia* twigs possessed fumigant toxicity against *L. bostrychophila* adults with an LC₅₀ value of 1.33 mg/l air while trans-cinnamaldehyde had an LC₅₀ value of 1.29 mg/l air (Table 2).

DISCUSSION

The main constituents of the essential oil of *C. cassia* oil were trans-cinnamaldehyde, acetophenone, trans-cinnamic acid and cis-cinnamaldehyde. In the previous studies, trans-cinnamaldehyde was always the main constituent compound in the essential oil of *C.*

cassia ranging from 30.36 % [6] to 87.5 % [8]. There were great seasonal and geographic variations in chemical composition of the essential oil of *C. cassia* because it has proved that there was variation in chemical composition of essential oil of *C. cassia* collected from different areas or from different parts of plants and also including the degree of maturity of the plant at harvest. For example, the essential oil of *C. cassia* observed at different stages displayed a different chemical profile [7]. The main compounds of the essential oils derived from different growth stages were trans-cinnamaldehyde (33.95-76.4 %), cinnamyl alcohol acetate (0.09-49.63 %), 2'-methoxy-cinnamaldehyde (0.09-6.69 %) and copaene (1.09-14.3 %). For practical use, it is necessary to standardize the essential oil of *C. cassia* twigs.

trans-Cinnamaldehyde and the essential oil exhibited strong contact toxicity against the booklice (Table 2). Compared with the positive control, pyrethrum extract ($LC_{50} = 18.99 \mu\text{g}/\text{cm}^2$), trans-cinnamaldehyde demonstrated only half level of acute toxicity against the booklice and the essential oil only 3 times less toxic. However, compared with the other essential oil constituents in the previous studies that were tested using a similar bioassay, trans-cinnamaldehyde possessed stronger contact toxicity against the booklice, e.g. estragole ($LD_{50} = 49.95 \mu\text{g}/\text{cm}^2$), trans-anethole ($LD_{50} = 57.98 \mu\text{g}/\text{cm}^2$) and fenchone ($LC_{50} > 156 \mu\text{g}/\text{cm}^2$) [18]. Moreover, the essential oil also has stronger acute toxicity against the booklice than the essential oil of *Foeniculum vulgare* because it was reported to have an LD_{50} value of $90.36 \mu\text{g}/\text{cm}^2$ [18].

trans-Cinnamaldehyde and the essential oil of *C. cassia* also possessed fumigant toxicity against *L. bostrychophila*. Compared with the positive control, dichlorvos ($LC_{50} = 1.35 \times 10^{-3} \text{ mg}/\text{l air}$), the crude essential oil and isolated constituent compound was almost 1000 times less toxic to the booklice, *L. bostrychophila*. In the previous reports, the essential oil of *C. cassia* showed contact and fumigant toxicity against the cigarette beetle (*L. serricornis*) [10], the rice weevils (*S. oryzae*) and the bean weevils (*C. chinensis*) [12]. At a dosage of $0.7 \text{ mg}/\text{cm}^2$, 100 % mortality of the three stored product insects was observed after 24 h exposure [11,12]. The essential oil of *C. cassia* also exhibited contact and fumigant toxicity against house dust mites (*Dermanyssus farinae* and *D. pteronyssinus*) [13]. Moreover, petroleum ether extract of *C. cassia* also exhibited acaricidal activity against house dust mites, *D. farinae* with an LC_{50} value of $4.6 \mu\text{g}/\text{cm}^2$ [19]. In addition, the isolated compound, trans-cinnamaldehyde was found to

have contact and fumigant toxicity against the oak nut weevil (*Mechoris ursulus*) [9], the rice weevils (*S. oryzae*) and the bean weevils (*C. chinensis*) [12]. The compound also exhibited contact and fumigant toxicity against the mites, e.g. house dust mites (*D. farinae* and *D. pteronyssinus*), the poultry red mite (*D. gallinae*) and the rabbit ear mite (*Psoroptes cuniculi*) (10; 13; 19; 20).

The above findings suggest that the contact and fumigant activity of the essential oil of *C. cassia* twigs and trans-cinnamaldehyde are quite promising. As currently used fumigants are synthetic insecticides and the most effective fumigants are also highly toxic to humans and other non-target organisms, the essential oil and trans-cinnamaldehyde show potentials to be developed as natural fumigants/insecticides for the control of booklouse. However, to develop a practical application for the essential oil and the isolated constituents as novel fumigants/insecticides, further research into the safety of the essential oil/compound to humans is needed. Additional studies on the development of formulations are also necessary to improve the efficacy and stability and to reduce cost.

CONCLUSION

The essential oil of *C. cassia* twigs and its constituent compound, trans-cinnamaldehyde demonstrated insecticidal activity against the booklice. However, further studies on safety of the essential oil and the isolated compound to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost.

ACKNOWLEDGEMENT

This work was supported by Special Fund for Agro-scientific Research in the Public Interest (grant no. 201003058). The authors thank Dr. QR Liu of College of Life Sciences, Beijing Normal University, Beijing 100875, China for the identification of the investigated medicinal herb.

REFERENCES

1. Turner BD. Psocids as a nuisance problem in the UK. *Pestic. Outlook* 1998; 9: 27-30.
2. Phillips TW, Throne JE. Biorational approaches to managing stored-product pests. *Ann. Rev. Entomol.* 2010; 55: 375-397.
3. Li XW. *Flora Reipublicae Popularis Sinicae*, Science Press, Beijing, Vol. 31, 1982; pp 223-226 [cited 2014 July 12]. Available from: http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200008698
Trop J Pharm Res, October 2014; 13(10): 1701

4. Jiangsu New Medical College. *Dictionary of Chinese Herbal Medicine*. Shanghai Science & Technology Press, Shanghai, China, 1977; pp 1771-1773.
5. Lockwood GB. The major constituents of the essential oils of *Cinnamomum cassia* Blume growing in Nigeria. *Planta Med.* 1979; 36: 380-381.
6. Wang R, Wang RJ, Yang B. Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. *Innov. Food Sci. Emerging Technol.* 2009; 10: 289-292.
7. Geng SL, Cui ZX, Huang XC, Chen YY, Xu D, Xiong P. Variations in essential oil yield and composition during *Cinnamomum cassia* bark growth. *Ind. Crops Prod.* 2010; 33: 248-252.
8. Qiu Q, Liu TL, Cui ZJ, Shen YM, Zhao Y. Study on chemical component of essential oil of *Cinnamomum cassia* Presl. by GC- MS. *Chin. J. Pharm. Anal.* 2000; 20: 248-251.
9. Park IK, Lee HS, Lee SG, Park JD, Ahn YJ. Insecticidal and fumigant activities of *Cinnamomum cassia* bark-derived materials against *Mechoris ursulus* (Coleoptera: Attelabidae). *J. Agric. Food Chem.* 2000; 48: 2528-2531.
10. Kim HK, Yun YK, Ahn YJ. Fumigant toxicity of cassia bark and cassia and cinnamon oil compounds to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. *Exp. Appl. Acarol.* 2008; 44: 1-9.
11. Kim SI, Park C, Ohh MH, Cho HC, Ahn YJ. Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). *J. Stored Prod. Res.* 2003; 39: 11-19.
12. Kim SI, Roh JY, Kim DH, Lee HS, Ahn YJ. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. *J. Stored Prod. Res.* 2003; 39: 293-303.
13. Kim SI, Kim HK, Koh YY, Clark JM, Ahn YJ. Toxicity of spray and fumigant products containing cassia oil to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *Pest Manag. Sci.* 2006; 62: 768-774.
14. Chang KS, Tak JH, Kim SI, Lee WJ, Ahn YJ. Repellency of *Cinnamomum cassia* bark compounds and cream containing cassia oil to *Aedes aegypti* (Diptera: Culicidae) under laboratory and indoor conditions. *Pest Manag. Sci.* 2006; 62: 1032-1038.
15. Adams RP. *Identification of Essential Oil Components by Gas Chromatograph /Mass Spectrometry*, 4th edn, Allured Publishing Corporation, Carol Stream, USA, 2007.
16. Sakuma M. Probit analysis of preference data. *Appl. Entomol. Zool.* 1998; 33: 339-347.
17. Laihia K, Pitkanen M, Nevalainen T. ¹³C-NMR study of aromatic ring-substituted (E)-3-phenylpropenals and (2E,4E)-5-phenylpenta-2,4-dienals. *Magn. Reson. Chem.* 1990; 28: 541-548
18. Zhao NN, Zhou L, Liu ZL, Du SS, Deng ZW. Evaluation of toxicities of some common spices essential oils from China against *Liposcelis bostrychophila*. *Food Control* 2012; 26: 486-490.
19. Wang ZQ, Kim HK, Tao WQ, Wang M, Ahn YJ. Contact and fumigant toxicity of cinnamaldehyde and cinnamic acid and related compounds to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *J. Med. Entomol.* 2011; 48: 366-371.
20. Shen F, Xing M, Liu L, Tang X, Wang W, Wang X, Wu X, Wang X, Wang X, Wang G, Zhang J, Li L, Zhang J, Yu L. Efficacy of trans-cinnamaldehyde against *Psoroptes cuniculi* in vitro. *Parasitol. Res.* 2012; 110: 1321-1326.