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Original/*Investigación animal*

Oribatid mite infestation in the stored Chinese herbal medicines

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Abstract

Objective: **to investigate the breeding status of oribatid mites and its related biological traits in the stored traditional Chinese herbal medicines.**

Methods: **sixty-three sorts of stored traditional Chinese herbal medicines of root and stem origins were collected from Huainan City in Anhui Province. The mites were isolated by Shakesieve and Tullgren, and identified and counted under the light microscope.**

Results: **thirteen samples in 63 were infested with the oribatid mites, which accounted for 21.67 %(13/60). A total of 11 species of oribatid mites, belonging to 6 families, were found in the 13 samples, and the most abundant species were *Scheloribates laevigatus* (36.19%), *Scheloribates latipes* (28.35%) and *Trhypochthpnius japonicus* (19.72%). The average breeding density of oribatid mites was about 4.51 individuals/g; the index of species richness, the diversity index, and the evenness index of species were 1.14, 1.581, and 0.212, respectively.**

Conclusion: **oribatid mites were found in stored Chinese herbal medicines from Huainan city in Anhui province of China, suggesting that the conventional storage and processing technique should be improved in order to ensure the quality and safety of the herbal medicines.**

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Key words: *Stored Chinese herbal medicines. Oribatid mite. Ecological parameters.*

INFESTACIÓN POR ÁCAROS EN EL ALMACENAMIENTO DE HIERBAS MEDICINALES CHINAS

Resumen

Objetivo: **investigar el estado reproductivo de los ácaros oribátidos y sus rasgos biológicos en el almacenamiento de medicinas a base de hierbas chinas tradicionales.**

Métodos: **sesenta y tres tipos de medicinas a base de hierbas chinas tradicionales extraídas de raíces y tallos almacenadas fueron recopilados en Huainan City, en la provincia de Anhui. Los ácaros fueron aislados por Shakesieve y Tullgren, y su identificación y recuento se realizó mediante microscopio de luz.**

Resultados: **trece de cada 63 muestras estaban infestadas por los ácaros oribátidos, lo que supone un 21,67% (13/60). Un total de 11 especies de ácaros oribátidos, pertenecientes a 6 familias, fueron encontrados en las 13 muestras, y las especies más abundantes fueron: *Scheloribates laevigatus* (36,19%), *Scheloribates latipes* (28,35%) y *Trhypochthpnius japonicus* (19,72%). El promedio de densidad reproductiva de los ácaros oribátidos fue de alrededor de 4,51 individuos/g; el índice de riqueza de especies, el índice de diversidad y la uniformidad del índice de especies fue 1,14, 1,581 y 0,212, respectivamente.**

Conclusión: **se detectó la presencia de ácaros oribátidos en las hierbas medicinales chinas almacenadas de Huainan City, en la provincia de Anhui, China, lo que sugiere que la técnica convencional de almacenamiento y procesamiento debe mejorarse a fin de garantizar la calidad y la seguridad de los medicamentos a base de hierbas.**

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Palabras clave: *Hierbas medicinales chinas almacenadas. Ácaros oribátidos. Parámetros ecológicos.*

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Introduction

Oribatid mites represent one of the common microarthropods, belonging to Arthropoda, Arachinida, Acari, Acariforms, Oribatida, and are found in species of variety. They vary a lot with respect to the morphological features, living habits, breeding materials, reproductive mode and places to breed in., and are known to include 800 genera under 134 families to date. Oribatid mites often breed on the soil surface, plant stems and leaves, and feed on and the organic matter from saprophytic materials and browse of vegetative covers[1]. Previous studies put more interest on the breeding status of oribatid mites in soils because of their special living habits[2-5], yet few reports are available on their infestation with the stored traditional Chinese herbs. From January to December of 2012, we conducted a survey on the breeding status of oribatid mites in 63 samples of traditional Chinese herbal medicines of rhizome origin collected from the storages in Huainan area of Anhui province, China. The current study was undertaken to report our findings.

Materials and methods

Instruments and materials

The apparatus and materials used for the experiment included: Sartorius analytical balance (BT214D, Sartorius AG, Germany), optical microscope (Nikon E-400, Japan), stereo microscope (Nikon SMZ-800, Japan), electric vibration sieve machine (85A-B, Fritsch CO. Ltd, Germany), electric heating device in mite (own product), sample screening sieve (ZNS-200, Beijing xingshi CO., China), permanent sealing agent (containing 10% 5 ml chloral hydrate, 20 ml glycerol, 30 g Arabic gum, and 50 ml distilled water), and absolute ethanol (analytical pure, Wuxi Zhangwang CO., China)

Methods

Sample collection

The samples were collected from the pharmacies and warehouses of traditional Chinese herbal medicines in Huainan area in Anhui province of China between June and August of 2012. All samples had been stored t for equal to or more than 6 months upon sampling. Three portions, weighing approximately 3 g, were taken from each sample, pouched separately and brought back to the laboratory. To understand the dynamics of oribatid mites, we also sampled the herbs three times every week by the first of week of each season from January through December of 2102, and the results were calculated on mean value.

Specimen isolation and identification

The samples were initially made to pass through a 40-mesh/per inch on a electrical vibrator to separate the residues. Oribatid mites in the major parts of the herbs were isolated with directicopy and Tullgren, while those in the residues were isolated with waternacopy and redricopy[6]. The specimens isolated were prepared for slide in accordance with the technique by Chaopin Li (1996)[7], identified under the light microscope for their morphology and counted. Classification of the oribatid mites was in compliance with the taxonomic system described by Krantz (1978)[8,9].

Data process

The ecological parameters were subjected to statistics for each samples, and calculated as the formula of $D = N/T \times 100\%$ for the breeding density (N stands for the number detected in each sample, T for the weight of the sample and D for the breeding density). The species richness index were represented by $R_{Margalef} = (S-1)/\ln N$ (S stands for the number of species, N denotes the total number of individuals in samples), species diversity index, by $H_{Shannon\text{-}Wiener} = -\Sigma Pi \ln Pi$ ($Pi = Ni/N$ is the probability of an individual belonging to the i species), and species evenness index, $J_{Pielou} = H'/Hmax$ ($Hmax = \ln S$).

Results

The breeding density of oribatid mites in different Chinese medicinal herbal samples and the classification

Higher breeding density of oribatid mites were observed in common yam rhizome (*Rhizoma Dioscoreae*), American Ginseng (*Radix Panacis Quinquefolii*), Radix Astragali (*Radix Astragali Mongolici*), white peony root (*Radix Paeoniae Alba*), and Abhesive rehamannia root tuber (*Radix Rehmanniae*). The results were listed in table I.

Thespecies of Oribatid mites breeding in the Chinese herbal rhizomes

Of the 63 samples, 13 were infested with the oribatid mites, in which 11 species belonging to 6 families were identified (see Table II). The most abundant species of oribatid mites were *Scheloribates laevigatus* (36.19%), *Scheloribates latipes* (28.35%) and *Trhypochthpnius japonicus* (19.72%).

Table I
The Density of Oribatid mites detected in the Chinese herbal medicines of rhizome origins

Samples	*Individual/g*	*Species of oribatid mites detected*
Rhizoma Dioscoreae	21.12	*Scheloribates laevigatus*, *Epilohmannia ovata*, *Trichoribates novus*, *Scheloribates latipes*
Rhizoma Belamcandae	9.78	*Scheloribates latipes*, Trhypochthoniidae Willmann, *Trhypochthonius japonicus*
Radix Saposhnikoviae	8.41	*Trichoribates novus*, *Eupterotegaeus armatus*
Radix Bupleuri Chinensis	8.85	*Scheloribates laevigatus*, *Scheloribates latipes*
Radix Salviae Miltiorrhizae	9. 12	*Trichoribates novus*, *Epilohmannia ovata*
Radix Rehmanniae	11.30	*Trichoribates novus*, *Scheloribates laevigatus*, ***Phanloppla lucorum***, *Trhypochthonius japonicus*
Rhizoma Pinelliae	8.30	*Scheloribates laevigatus*, *Trichoribates novus*
Rhizoma Polygonati Sibirici Delar	8. 65	*Scheloribates laevigatus*, *Trhypochthonius japonicus*
Radix Ophiopogonis Japonici	8.18	*Scheloribates laevigatus*
Radix Paeoniae Alba	12. 28	*Scheloribates laevigatus*, *Trichoribates novus, Hypochthonius minutissima*, *Scheloribates latipes*
Radix Astragali Mongolici	15. 16	*Trichoribates novus*, *Scheloribates laevigatus*, *Trhypochthonius tectorum*, *Trhypochthonius japonicus*
Radix Ginseng	9.68	*Scheloribates laevigatus*, *Epilohmannoides terrae*
Radix Panacis Quinquefolii	18.32	***Phanloppla lucorum*****,** *Trichoribates novus*, *Microzetes auxiliaries*, *Scheloribates latipes*

The ecological parameters of Oribatid mites in different sorts of the Chinese herbal medicines

In 63 herbal samples, the average breeding density of oribatid mites was approximately 4.51 individuals /g, the index for species richness, diversity and species evenness were 1.14, 1.581 and 0.212, respectively. The highest breeding density of oribatid mites was associated with common yam rhizome (*Rhizoma Dioscoreae*), American Ginseng (*Radix Panacis Quinquefolii*), Radix Astragali (*Radix Astragali Mongolici*), white peony root (*Radix Paeoniae Alba*), and Abhesive rehamannia root tuber (*Radix Rehmanniae*). And the details were listed in table III. The results showed that the ecological habits and habitats varied a lot for diverse species of the oribatid mites, since the highest

Table II
The species of Oribatid mites breeding in Chinese herbal medicines of rhizome origins

Family	*Genus*	*Species*
Scheloribatidae Grandjean	*Scheloribates*	1. *Scheloribates laevigatus*
		2. *Scheloribates latipes*
	Trichoribates	3. *Trichoribates novus*
Trhypochthoniidae Willmann	*Trhypochthonius*	4. *Trhypochthonius japonicus*
		5. *Trhypochthonius tectorum*
Epilohmanniidae Oudemans	*Epilohmannia*	6. *Epilohmannia ovata*
		7. *Epilohmannoides terrae*
Eniochthoniidae Grandjean)	*Hypochthoniu*	8. *Hypochthonius minutissima*
Microzetidae Grandjean	*Microzetes*	9. *Microzetes auxiliaris*
Cepheidae Berlese	*Eupterotegaeus*	10. *Eupterotegaeus armatus*
		11. *Phanloppla lucorum*

Table III
The ecological parameters of Oribatid mites in different herbal rhizomes

Ecological parameters	*Herbal categories*				
	Rhizoma Dioscoreae Oppositae	*Radix Panacis Quinquefolii*	*Radix Astragali Mongolici*	*Radix Paeoniae Alba*	*Radix Rehmanniae*
Breeding density	21.12	18.32	15.16	12.28	11.30
Richness index	1.16	1.19	1.04	0.76	1.82
Diversity index	1.49	1.26	1.23	1.61	1. 96
Evenness index	0.83	0.81	0.79	0.86	0.91

mite breeding density was found in white peony root (*Radix Paeoniae Alba*), and the maximum richness index, diversity index and evenness index were seen *Radix Rehmanniae,* which suggested that the Chinese medicinal herbs of root and and stem origins are prone to infestation with oribatid mites rich in species diversity, yet the categories of species remain relatively stable.

Seasonal distribution for the oribatid mites in the stored herbal medicinal rhizomes

We also examined the seasonal distribution of oribatid mites in the Chinese herbal medicines, and the the trends of community parameters are shown in table IV.

Discussion

Oribatid mites are important species in terrestrial ecosystems, and play significant part in decomposition processes of the organic matter and nutrients cyclingand in soil formation[11,12], for which they are recognized as optimal indicators for monitoring over the quality of terrestrial soils. Interestingly, our work indicates that oribatid mites also occur in the stored traditional Chinese medicinal herbs with variety of diverse species. We observed a relatively higher breeding density (4.5.1 individuals/g) and richness index (1.14) of oribatid mites in the samples we collected. This showed that the stored traditional Chinese herbal medicines are seriously infested with such mites, and the breeding species are consistent with the distribution and species in Anhui, China, reported by Chaopin Li[9]. Currently, the harm of oribatid mites starts to draw the attention of researchers with emerging research on this species. Xiaodong Zhan, Qinggui Yang *et al*.[13,14] had reported the occurrence of oribatid mites in the Chinese herbal medicines, which were also detected in current work. What's more, oribatid mites are recognized as potential intermediate hosts of Anoplocephalidae and attributable to spreading of certain diseases[15-17]as well as do harm to the economic crops. Therefore, it is essential and urgent to make a scientific evaluation on the influence of oribatid mites on environment and stored products.

Our investigation revealed that the stored traditional Chinese medicinal herbs infested with oribatid mites are origins of plant rhizomes. This may be associated with incomplete dusting the soil residues off the plants in processing, resulting in breeding of the oribatid mites in the herbal roots. Hughes (1976) considered that breeding of the oribatid mites indoors or in the stored products were the passage through windows or human carriers[1]. However, as far as the habitat traits and omnivorousness of oribatid mites are concerned, the Chinese medicinal herbs together with its storage environment are abundant food resources and favorable to their living. Therefore, improvement on the purity of the Chinese herbal medicines in processing and storage conditions can ensure extended storage time and quality of the products, because of certain selectivity of oribatid mites with the temperature, humidity and food resources[18]. Anhui province lies in the region of East Chinaat the lower reaches of the Yangtze River,

Table IV
Variation trends of community parameters for oribatid mites in the four quarters

Months	*Average breeding density (Individuals/g)*	*Richness index*	*Diversity index*	*Evenness index*
Jan.	6.53±2.29	2.113±0.036	1.315±0.212	0.918±0.042
Apr.	5.47±2.80	1.432±0.118	1.066±0.115	0.935±0.036
Jul.	9.32±2.33	6.013±0.021	3.139±0.084	0.791±0.059
Oct.	11.17±2.12	5.418±0.011	2.738±0.091	0.801±0.023

and has larger span between the north and south border. Subtropical humid monsoon climate in Anhui area leads to abundant rainfall and distinct four seasons. The annual average temperature is between 15ºC and 16ºC, and yearly average rainfall, 1200 mm, especially the average temperature in summer and autumn seasons reaches 29ºC to 33ºC, and the humidity is some 68%-76% in those two quarters, which makes optimal conditions for the orbited mites to reproduce in large quantity. Chaopin Li *et al*.[19] investigated the oribatid mite community and its diversity, and found that the average breeding density and species richness as well as diversity were higher in the south Anhui areas than in Huaibei plain, Jianghuai hilly region and central plain.

We examined the average breeding density of oribatid mites in Jan. Apr. Jul. and Oct., respectively, in the stored Chinese medicinal herbs in Huainan area, and found that 13 of 63 samples were infested with the oribatid mites. Maximal average breeding density was observed in Common Yam Rhizome, followed by American, and Mongolian Milkvetch Root. In order to understand the seasonal distribution of oribatid mites in the stored traditional Chinese herbal medicines, we performed a calculation and analysis on the average breeding density, community diversity and index of richness and evenness regarding the samples collected in the same storage in different seasons, and found that the index of richness and diversity were highest in July, whereas October represented maximal average breeding density, and April the top evenness index. Previous reports described that the breeding of oribatid mites were affected by temperature, humidity, lighting conditions, their feeding habits and human disturbance[20]. This is consistent with our findings. In our sampling in July, when the average temperature in the warehouse was 31ºC with 75.6% RH, the storage was tightly closed with poorer ventilation and without regular cleaning, which resulted in reproduction of the oribatid mites in large quantity. In October when the temperature was 25ºC with 66% RH, the index of richness and diversity appeared fallen somewhat, yet the breeding density was maximum. This may be associated with longer breeding periods of the oribatid mites, since many females lay eggs in summer season and reproduction occurs in autumn. If the human disturbance is excluded, we consider that average breeding density, index of diversity, community richness and evenness for the oribatid mites are greatly involved in the ambient temperature and humidity, because related highest indicators were seen in summer season[21].

We only identified part of the oribatid mites in some of the samples without statistics for remaining species. Meanwhile, it is hard to completely isolate the species from the samples. Therefore, the real count of oribatid mites in the samples might be over our findings. Apart from that, we estimated the breeding quantity of oribatid mites in full sample size exclusively by indirect calculation of the density; this may by and large represent the density of oribatid mites in each herbal sample. Nevertheless, our findings may supply evidence for management of the stored traditional Chinese medicinal herbs and prevention of the oribatid mites breeding in scientific manner, yet research of the oribatid mites regarding their molecular biology, molecular genetics and physiology and pathology remains necessary, which is of great significance for the soil ecological protection or preservation of the stored products.

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