

Original article

# Distribution of arthropods in rice grains in Malaysia

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## Abstract

**Objective:** To determine distribution of arthropods in rice grains obtained from different sources. **Methods:** Rice samples were randomly collected from public in urban areas, farmers in rice field areas, aborigines in undeveloped areas and retailers in commercial premises. Random samples of rice were taken out from each sample for isolation of arthropods using a modified Berlese Tullgren Funnel Method. Mites were mounted prior to identification; weevils were directly identified. **Results:** Samples of rice from retailers in commercial premises had the highest infestation by arthropods followed by samples from urbanites, aborigines and rice farmers. Two species of weevils, *Sitophilus oryzae* (*S. oryzae*) and *Sitophilus granarius* (*S. granarius*), were found. Samples from commercial premises had the least percentage of weevils compared to those collected from domestic premises. Depending on the source of samples, densities of *S. granarius* and *S. oryzae* ranges from 11-103 weevils/kg and 7-80 weevils/kg, respectively. Important species of mites in stored rice identified were mainly members of the families Cheyletidae, Echimyopodidae, Pyroglyphidae, Saprogllyphidae and Tenuipalpidae. Among the species of mites identified were *Austroglycyphagus malaysiensis*, *Cheyletus fortis*, *Cheyletus malaccensis*, *Dermatophagoides pteronyssinus*, *Grammolichus malukuensis* and *Suidasia pontifica*. Average density of most of the mites was less than 40 mites/kg of rice grains. In this study, the highest number of mites in rice samples was recovered from commercial premises, followed by samples from urbanites. Samples from farmers and aborigines contained lesser mites. **Conclusion:** This study demonstrated the presence of 3 allergenic mite species in rice, i. e. *A. malaysiensis*, *D. pteronyssinus* and *S. pontifica*. Weevils, *S. oryzae* and *S. granarius* that are known to be allergenic, were also found.

**Keywords:** Distribution; Arthropods; Rice grains

## INTRODUCTION

Rice (*Oryza sativa*) is the main staple food for more than half of the world's population and is of great economic importance<sup>[1]</sup>. In Malaysia, an average person consumes about 420 g of rice daily<sup>[2]</sup>, accounting for 33 % of total calorie intake<sup>[3]</sup>. Thirty percent of adult Malaysians with rhinitis had positive

skin prick test to rice allergen<sup>[4]</sup>. However, stored rice grains may contain many allergens. The allergens may be from rice grain itself<sup>[5]</sup>, dead bodies and excretion of arthropods such as mites and weevils that infest rice<sup>[6-8]</sup> and from fungi on rice and mites<sup>[9-11]</sup>.

Individuals who consume unprocessed or processed food infested with the above arthropods are at risk of digestive problems and may exhibit symptoms such as asthma, rhinitis and/or allergic dermatitis which in extreme cases, may lead to anaphylaxis<sup>[12,13]</sup>. Prolonged contact with infested foods may produce a mild dermatitis.

The importance of mite and weevil-induced aller-

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gies caused by the presence of these arthropods and their secretion in rice is still unknown in Malaysia. Since rice is the main staple food for Malaysians, there is a potential risk of allergic reactions in individuals who come into contact with stored rice. The distribution of mites and weevils in stored rice has not been reported in Malaysia. The purpose of this study is thus to document the presence of arthropods in stored rice from various sources in Malaysia.

## MATERIALS AND METHODS

Samples of rice were randomly collected from public in urban areas, farmers in rice field areas, aborigines in undeveloped areas and retailers in commercial premises. Localities of rice collection are shown in Figure 1. Samples of rice from 150 public were collected from Klang Valley ( $n = 30$ ), Selangor ( $n = 30$ ), Seremban ( $n = 30$ ), Johor Bahru ( $n = 30$ ) and Port Dickson ( $n = 30$ ). Samples from commercial premises were collected from various grocery shops and mini-markets that sell rice in loose forms in gunny ( $n = 108$ ) or plastic sacks ( $n = 42$ ).



**Figure 1** Sketch map showing localities where rice samples were collected.

Rice samples were also collected from 150 farmers in rice field areas in Kuala Selangor, Tanjung Karang

and Sekinchan. These samples were stored either in special large man-made wooden containers, locally known as *kepek* (63.0 %), large water drums (19.0 %), gunny sacks (13.0 %) and big earthen pots that are locally known as *tempayan* (5.0 %). One hundred and fifty (150) samples were collected from seven aborigine settlements in Kemidak-Selai, Endau Rompin, Johor ( $n = 33$ ); Pasoh, Jelebu, Negeri Sembilan ( $n = 26$ ); Kuala Lah ( $n = 20$ ), Sungai Lah ( $n = 12$ ) and Sungai Rual ( $n = 10$ ), Gua Musang, Kelantan; Pos Raya, Simpang Pulai, Kinta, Perak ( $n = 32$ ); and Pos Chiong, Sungai Banon, Gerik, Perak ( $n = 17$ ). The aborigines purchased their rice.

A random sample of rice, weighed  $260 \pm 2.0$  gram was taken out from its original container using a standard 250 mL plastic beaker. All samples collected were in use during the period of study. Isolation of arthropods was done using a modified Berlese Tullgren Funnel Method. A sub-sample of 150 grams of each rice sample was introduced into a 12 cm diameter sieve with a 1 mm mesh size that was placed approximately 7.0 cm below a lighted 60 watts frosted light bulb. A 14 cm diameter petri dish containing concentrated lactic acid was placed directly below the sieve to collect arthropods displaced from the rice. The lighted bulbs were switched on for three continuous days or until no more arthropods were found. Petri dishes were checked for arthropods twice a day. The whole process was repeated using the same sample after 1 week to ensure complete extraction of arthropods inclusive of newly hatched larval stages.

The techniques for preparation of mites for identification followed those used by Ho & Nadchatram<sup>[14]</sup>. Mites collected were suspended in 90 % lactic acid and then heated with constant stirring. The heat source was removed as soon as the suspension began to bubble. The suspension was left to cool, after which a small volume at a time was transferred to a Petri dish and examined under  $20 \times$  magnification. Whole mites and mite fragments were picked up with a sharpened applicator stick and were mounted in Hoyer's medium. Mounted slides were dried in an oven at  $40 \text{ }^\circ\text{C}$  for 10-12 days before the mites were identified. Weevils were picked up manually and identified at  $20 \times$  magnification. Data analysis was carried out using the statistical package SPSS version 11.5 for windows. All statistical analyses were performed at 95 % confidence level. Analysis of variance (ANOVA) and student's *t*-test were

used for multiple comparisons of means and for comparison of 2 means, respectively.

## RESULTS

### Distribution of arthropods in rice from public in urban areas

Infestation of arthropods occurred in 25.3 % of the samples (Table 1). The samples were either infested only by weevils (19.3 %), mites (2.0 %) or both weevils and mites (4.0 %). Weevils of the family Curculionidae were more commonly found in samples (94.0 %) compared to mites (6.0 %) (Tables 2 & 3).

Two species of weevils were found i. e. *S. oryzae* Linnaeus (61.3 %) and *S. granarius* Linnaeus (10.3 %). The former is commonly known as rice weevil and the latter as grain weevil. An average of 80 live *S. oryzae* and 17 live *S. granarius* were found in a kilogram of rice grains, respectively. About 27.0 % of the weevils found were larval stages. Density of the immatures was 31 weevils/kg of rice grains. Common mites found include *Cheyletus fortis* (4.8 %), immature *Cheyletus* spp. (4.8 %) and *Dermatophagoides pteronyssinus* (4.8 %). Others were composed of a high percentage of free living mites (42.9 %) and those of the family Tenuipalpidae (4.8 %). *Dermatophagoides pteronyssinus* is a well-known allergens producing dust mite in Malaysia [15-22].

Majority of rice samples (64.0 %) were kept in their original packages, i. e. either plastic bags (61.0 %) or gunny sacks (3.0 %). Other storage containers used were 5 gallons plastic drum with cover and other plastic wares (32.0 %), various sizes of metal bins (3.0 %) and commercial rice dispensers (1.0 %). Eighty six percent (86 %) of the rice was kept in closed containers and 14.0 % was in opened containers. Arthropods infestation rates in closed and opened container were 37.0 % and 43.0 %, respectively; the difference is not significant ( $P > 0.05$ ).

Rice containers were stored mainly in kitchens (93.0 %) and store rooms (7.0 %). Most of the container (88.0 %) was kept on the floor (67.0 %) inside cabinets (21.0 %), on tables (5.0 %), under tables (3.0 %), under kitchen sinks (2.0 %) or inside refrigerators (2.0 %). Rice kept in different storage places had similar infestation rates ( $P >$

0.05).

### Distribution of arthropods in rice from retailers in commercial premises

Infestation of arthropods occurred in 31.3 % of the samples (Table 1). The samples were mainly infested by mites (18.0 %), followed by weevils (10.0 %). Infestation by both weevils and mites occurred only in 3.3 % of the samples. Percentage of samples infested by weevils and mites were about similar i. e. 42.3 % and 57.7 %, respectively (Tables 2 & 3). Weevils found were represented by 2 common species, *S. granarius* (37.3 %) and *S. oryzae* (3.6 %). An average of 103 live *S. granarius* and 7 live *S. oryzae* were found in a kilogram of rice grains. Twenty five percent (25.0 %) of unidentified weevils were immature stages with the density of 25 immatures/kg of rice grains.

The most abundant mites found were *Cheyletus malaccensis* (14.2 %), followed by *Cheyletus fortis* (7.1 %). Immatures *Cheyletus* spp. represented 76.0 % of mites found in rice from the commercial premises and this contributed to a total of 97.3 % of total mites belonging to the genus *Cheyletus*. Other mites found were *Austroglycyphagus malaysiensis* (0.9 %), *Grammolichus malukuensis* (1.8 %) and *Suidasia pontifica* (0.9 %). *Austroglycyphagus malaysiensis* and *S. pontifica* are known to produce allergens [23,24]. Majority of rice sold in these premises were in their original packaging (93.0 %) either in 50 kg jute gunny sacks (55.0 %), 50 kg plastic sacks (17.0 %) or in 10 kg plastic bags (21.0 %). Rice in these sacks and bags were displayed openly. The balance of the rice samples (7.0 %) were in closed 5 gallon plastic drums. Rates of infestations were significantly different between packaging ( $P = 0.018$ ). Rice in jute gunny sacks was most infested.

Fifty one percent (51.0 %) of the rice samples were placed in the middle area of the shop while the other 31.0 % and 18.0 % were kept at the back and front, respectively. The occurrence of infestation rates in different areas of the shops were found to be similar ( $P > 0.05$ ). Forty nine percent (49.0 %) of the rice containers were kept in bright area, 23.0 % in shadows and (28.0 %) in the dark. Infestation occurred irrespective of the level of lighting. However, the lighting had no significant effect on the infestation rate ( $P > 0.05$ ).

**Table 1** Number of rice grains samples infested by arthropods[  $N(\%)$  ].

Source of rice grains	N	Number of samples*		Number of samples infested by arthropods*		
		Clean	Infested	Weevils	Mites	Weevils + mites
Urbanites	150	112 (74.7)	38 (25.3)	29 (19.3)	3 (2.0)	6 (4.0)
Retailers	150	103 (68.7)	47 (31.3)	15 (10.0)	27 (18.0)	5 (3.3)
Farmers	150	138 (92.0)	12 (8.0)	10 (6.6)	1 (0.7)	1 (0.7)
Arborigines	150	124 (82.7)	26 (17.3)	25 (16.7)	0 (0.0)	1 (0.7)

\* Percentage in parentheses

**Table 2** Weevils identified in rice grains from different sources[  $N(\%)$  ].

Source of rice grains	N	Number of weevils recovered*			Number of weevils identified*			Average densities ( number/kg rice )		
		Adult	Immatures	Total	<i>S. granarius</i>	<i>S. oryzae</i>	<i>S. granarius</i>	<i>S. oryzae</i>	Immatures	
Urbanites	150	237 (67.3)	94 (26.7)	331 (94.0)	34 (10.3)	203 (61.3)	17	80	31	
Retailers	150	34 (17.3)	49 (25.0)	83 (42.3)	31 (37.3)	3 (3.6)	103	7	25	
Farmers	150	21 (2.1)	956 (97.7)	977 (99.8)	16 (1.6)	5 (0.5)	11	33	1275	
Arborigines	150	115 (17.8)	531 (82.1)	646 (99.9)	96 (14.9)	19 (2.9)	38	16	393	

\* Percentage in parentheses

**Table 3** Mites identified in rice grains from different sources.

Source of rice grains	N	Number of mites recovered* ( $N, \%$ )	Mites identified*	Average densities ( number/kg rice )
Urbanites	150	21 (6.0)	<i>Cheyletus fortis</i> (4.8 %)	7
			<i>Cheyletus</i> spp. (4.8 %)	7
			<i>Dermatophagoides pteronyssinus</i> (4.8 %)	7
			Free living mites (42.9 %)	12
			Tenuipalpidae mites (4.8 %)	7
			Distorted mites (38.1 %)	13
Retailers	150	113 (57.7)	<i>Austroglycyphagus malaysiensis</i> (0.9 %)	7
			<i>Cheyletus fortis</i> (7.1 %)	13
			<i>Cheyletus malaccensis</i> (14.2 %)	12
			<i>Cheyletus</i> spp. (76.0 %)	36
			<i>Grammolichus malukuensis</i> (1.8 %)	13
			<i>Suidasia pontifica</i> (0.9 %)	7
Farmers	150	2 (0.2)	Free living mites	7
Arborigines	150	1 (0.1)	Free living mites	7

\* Percentage in parentheses

### Distribution of arthropods in rice from farmers in rice field areas

Only 8.0 % of the samples were infested ( Table 1 ). Weevils were the main arthropods ( 99.8 % ) ; 2 species were present: *S. granarius* ( 1.6 % ) and *S. oryzae* ( 0.5 % ) ( Table 2 ). An average of 11 live *S. granarius* and 33 live *S. oryzae* were found in a kilogram of rice grains, respectively. Most of the weevils ( 97.7 % ) were in the immature stages with the density of 1 275 immatures/kg of rice grains.

Unidentified free living mites of the family Tarsonemidae were found only in 2 samples.

Sixty three percent of the rice ( 63.0 % ) was stored in special large man-made wooden containers ( *kepuks* ). Majority of *kepuks* were kept in the kitchens ( 89.0 % ). Some *kepuks* were placed in special buildings or places ( 11.0 % ) either as separate units outside the house or under the houses. Other types of container used were large water drums ( 19.0 % ) ; gunny sacks ( 13.0 % ) and big earthen



pots (*tempayan*) (5.0 %). Rice kept in different containers had similar infestation rates ( $P > 0.05$ ).

### Distribution of arthropods in rice from aborigines in undeveloped areas

Only 17.3 % of the samples were infested with arthropods (Table 1). Weevils were the most commonly found (99.9 %) and represented by 2 common species, *S. granarius* (14.9 %) and *S. oryzae* (2.9 %) (Table 2). An average of 38 live *S. granarius* and 16 *S. oryzae* were found in a kilogram of rice grains. Immature stages of weevils represented 82.1 % of total weevils found with the density of 393 immatures/kg. Unidentified free living mites contributed 0.1 % of the infestation. Information on the storage places was not available due to the lack of co-operation from the individual aborigines and as most samples were collected by the community chief or *Tok Batin*.

## DISCUSSION

Infestation of stored rice by arthropods (mites and/or weevils) is a common problem in Malaysia and world wide. Samples of rice from retailers in commercial premises had the highest infestation rate compared to those from domestics. For the latter, rice from people in the urban areas contained highest number of arthropods, followed by those from the aborigines and rice farmers. Rate of infestation of rice from commercial premises found in this study was almost similar to those recovered from markets in Sao Paulo where 31.7 % infestation was reported [25].

In this study, rice grains were mainly infested by weevils (Coleoptera: Curculionidae) and mites (Acari). Two species of weevils were found i. e *S. oryzae* and *S. granarius*. They are the most important destroyers of stored whole rice grain in commercial premises in many parts of the world [26]. However, in this study, samples of rice from commercial sources had the lowest infestation rate by weevils compared to samples from the domestic sources. This agrees with a report that domestic environment is an important factor in developing infestation [27]. The faster turn-around time in commercial compared to domestic premises is another possible reason for the difference of infestation rate by weevils.

Average densities of *S. granarius* were 15-fold and 2-fold higher than *S. oryzae* in samples of rice from the commercial premises and the aborigines, respectively. On the other hand, *S. oryzae* was more

dominant in samples from urbanites and farmers. A report that claimed the major source of *S. oryzae* are small packages of rice [28] is therefore not anymore relevant as the farmers kept their rice grains in a bulk amount of more than 100 kg. Comparing these 2 species, *S. granarius* is a much more destructive pest of stored products compared to *S. oryzae* [29].

*S. granarius* and *S. oryzae* have been demonstrated to be allergenic respiratory allergens [6,7]. However, skin prick test (SPT) was conducted only for *S. oryzae* extracts and it showed 38.5 % of positive rates in inhabitants of a building infested by the species [6]. A study on sensitization and allergic reactions to unidentified species of *Sitophilus* weevils has been conducted in Malaysia. Extract of the species produce positive SPT reactions in 40.9 % and 28.6 % of rice millers and farmers, respectively [30].

Important species of mites in stored rice identified from this study are mainly members of the families Cheyletidae, Echimyopodidae, Pyroglyphidae, Saprogllyphidae and Tenuipalpidae. Among the species of mites identified were *A. malaysiensis*, *C. fortis*, *C. malaccensis*, *D. pteronyssinus*, *G. malukuensis* and *S. pontifica*. Most of the samples positive for mites had average densities of less than 40 mites/kg of rice grains. In this study, the highest number of mites in rice samples was recovered from the commercial premises, followed by samples from the urbanites. Samples from the farmers and the aborigines contained lesser mites.

The species of mites recovered from these rice samples, were also found in house dust in Malaysia [14,22,31]. Among the mites found in this study, three species are important mites causing allergic reactions. The species were *A. malaysiensis*, *D. pteronyssinus* and *S. pontifica* [22-24,32,33]. Ingested allergens in mite-contaminated food are an emerging food safety issue as the allergens may induce systemic anaphylactic reactions in patients with respiratory allergy to mites [12].

This study showed that most infestation of mites took place in commercial premises where rice was displayed openly. Infestation is most likely to occur in sacks and packages that have been opened for removal of a portion of its contents and then left unsealed for long periods of up to two or three months. Nature of display and where the gunny or plastic sacks were placed may be contributing factors affecting infestation. Most of the rice containers or sacks on display were placed near to other stored grains and this may contribute to cross-contamination or

cross-infestation. Most rice sold in 50 kg gunny sacks were found to be of lower quality with many broken kernels and rice dust. These broken kernels may probably allow mites to lay eggs easily without making any bores in the grains. Fine grains and rice dust can be used as an indicator for mite infestations as they cannot feed on unbroken kernels [34].

Plenty of food available may support the development and reproduction of mites. This was shown by the high number of mite species extracted out from commercial premises. There are great possibilities that mite abundance will attract more predators. This study showed a high number of *Cheyletus* was found in the commercial premises where most number of mites was found. The two species of *Cheyletus* found were those commonly found in house dust [22]. In this study, *C. malaccensis* was recorded as the most common Cheyletids and this is in accordance with the study of mites associated with stored products [35]. *Cheyletus malaccensis* has been reported to cause localized skin problems in humans [36].

Infestation rate of arthropods in rice samples from the aborigines was low because their stock of rice is low and manageable. Amount of rice stocks was found to be not more than 5 kg at any particular time. Some of them do not keep any rice at home. Generally, the staple foods for most aborigines are mainly their own grown tapioca, sweet potatoes and bananas. Personal communications with some of the aborigine community chiefs showed that they seldom eat rice because of money constraints and transport difficulties. Settlements of aborigines visited were in undeveloped areas. Rice was only purchased in a nearby town when they went out to deal or sell forest commodities.

Samples of rice from the farmers were the least infested. This is possibly due to a shorter duration of storage. All samples from farmers were newly harvested grains and were less than a month when the sampling was done. Most farmers did their own traditional milling whenever they need rice stock for their consumption.

Information gained from this study provides evidence for the prevalence of allergenic mite and weevil populations in rice. As with mites, extract of weevils should be considered for diagnostic testing and possible immunotherapy. To avoid under-diagnosed, it is important to know which mite or weevil species are present in an area when performing diagnostic testing. This study showed that infestation of rice by mites and weevils occurred regardless of

sources of the rice. This study confirmed the importance of improving rice storage condition in commercial and domestic premises to avoid mites and weevils infestation.

The uses of botanic materials as traditional ways of avoiding infestation of arthropods were observed throughout the collection of rice samples. Dried red chilly, *Capsicum annum* was among the most common materials seen kept together with the rice grains inside the storage containers. Besides that, dried black pepper seeds, *Piper nigrum* wrapped in muslin cloths were also observed in the piles of rice grains. Dried leaves of *Averrhoa bilimbi* (*belimbing buluh*); *Pandanus* spp. (*pandan*); *Tamarindus indica* (*asam jawa*); *Psidium guajava* (*jambu batu*); *Citrus hystrix* (*limau purut*) and *Citrus aurantifolia* (*limau nipis*) were those used by people living in rural and remote areas. There is little or no information on evaluation of these materials as repellants against infestation of arthropods in rice grains in Malaysia. A further study is therefore recommended to determine repellent effect of the materials.

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## Disclaimer

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