Frequency and diversity of pest arthropods in stored cereals in a military unit

Khoobdel M.1 PhD, Ma’rouf A.2 PhD, Farajzadeh D.* PhD, Vatani H.3 MSc, Riazipour M.3 PhD, Joneydi N.1 MD

* Health Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran; 1 Health Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran; 2 Department of Agricultural Entomology, Research Institute of Plant Protection, Agricultural Research, Education & Extension Organization, Tehran, Iran; 3 Department of Parasitology & Mycology, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

Abstract
Aims: Military Forces have to store primary food ingredients in order to have food security and provide foods for their personnel. Therefore detection of potential pests in warehouses can be very important in their control and maintenance of food. The aim of this study was to determine the abundance and diversity of pests and also the rate of cereal contamination in the warehouses of military units.

Methods: This cross-sectional study was done during 2007-9 in 9 military grain storages. Sampling of cereals was performed according to the method of Standards Institute and Industrial Research of Iran. Separation of arthropods from cereals and beans was performed by direct observation, using the stereomicroscope, Berlese funnel, flotation, vapor exposure and etc. Data was analyzed by descriptive and analytical statistical methods by SPSS 12 software.

Results: Four species of pests including Oryzaephilus mercator, Tribolium castaneum, Bruchus lentis and Bruchus rufimanus were determined and respectively separated from rice, barely, lentil and broad bean. The warehouse 1 and 3 were the most contaminated places, respectively with mean 1.3±0.65 and 1.4±0.6 of grain per 100g. Barley with about 3.2±1.1 per 100g was more contaminated than other products.

Conclusion: Contamination of cereals in the studied warehouses is often less than standard and in only two cases of barely stocks is more than limited.

Keywords: Storage Pests, Arthropod Pests, Cereals, Food Storage

Introduction
Arthropods are a huge group of invertebrates that have bodies and articulated legs. The characteristics of all arthropods in larval and maturity are circles and articulated legs in their physiologic structure. Insects are the main categories in the branches of arthropods [1]. Storage pests are mostly animals that enter the warehouses and spoil the food storage qualitatively and quantitatively [2]. Moreover, they might cause health problems and gastrointestinal disorders for all users [3]. Some of the arthropods that are accustomed to the condition of warehouse life and feeding from cereal are regarded as the most significant storage pests [4]. Storage pests destroy 10-20% of agricultural products annually and even in some countries that don’t have proper conditions for keeping products in warehouses or those that face with natural disasters, this amount might increase to 50%. All the countries face with storage pests and international business of food products makes the extent of contamination wider [5]. Depending on the type of arthropod, contamination of food to storage pest might happen on the farm or the warehouse from the beginning and in most of the cases, each type of arthropods can infect one type of the product [4]. More than 150 types of storage arthropods pests have already been recognized, among which types such as Sitophilus oryzae, Tribolium and bean beetle are highly important. These arthropods are often among the categories of insects, and from the order of Coleoptera, Lepidoptera, and Hemiptera. Among the most important storage pests in Iran, one can refer to two types of Tribolium, Callosobruchus maculatus, Trogoderma granarium, Rhizopertha dominica, and Ephesia kuehniella and some other types [7, 8, 9, 10].

Owning healthy food storage to supply soldiers on time, is regarded as the most important army forces. Therefore, buying and storing food products including cereals are inevitable for them. In this regard, recognizing probable storage pests might be important to decrease, control, and keep food products for military units both economically and hygienically.

Since Iranian researches face with some problems to access the military warehouses, the studies on storage pests especially in military warehouses are very
limited, and no study has been published on this issue yet. However, good studies as well as good laboratory studies have been performed in other food warehouses of the country [3, 4, 5, 6, 7, 8, 9, 10, 11], such as analysis of storage pests of grain elevators to recognize the arthropods of grain pest in some provinces of Iran such as Tehran, Golestan, and Kermanshah [3] as well as fanatic study of storage pests insects in some cities of Golestan province [11] to name a few. Various studies have been performed on natural occurrence of storage pests in agricultural products and the food products in other countries [12, 13, 14, 15, 16, 17].

The aim of this study was the analysis of frequency and variety of types, as well as the amount of contamination of cereals in food storage of one of the commands of military units based in Tehran.

**Methods**

This cross-sectional study was performed in 2007-2009 that was performed for 2 years in cereal and grain warehouse units of one of the military units based in Tehran and entomology laboratories of institute for plan research of Iran and the center for military health research of one of the medical universities of Tehran. In accordance with the aim of study, the parts of grain and cereal warehouses were sampled as the research community.

**Sampling:** First and before anything else, the necessary coordination was done to access the food of the mentioned military unit based in Tehran. To do the sampling from the regarded storage products, type and total amount of grains and cereals of storages were determined. Then, according to the labor regulation, no. 2087 and no. 3735 of Standard Institute and Industrial Researches of Iran (the sampling method of grains), the grains and cereal of the warehouses were sampled according to their inventory [17, 18]. Times and types of sampling and amount of the sample were determined by the use of mentioned standard. In order to do that, the sample bags were numbered and marked by the use of table of random numbers. The sampling was performed twice in every year in spring and summer (2007, 2008), and the sampling was performed 4 times from each product in every storage. It should be mentioned that according to the instruction of plant protection organization, department of pests’ control, if the number of cereals and grains were among 1-10 in the warehouses all the bags were sampled. Moreover, if there were 11-100 bags, 10 bags were sampled randomly. In the cases higher than 100 bags, the sampling was done by the square root of rounded numbers [18].

In order to do that, the sampling was done from 3 different spots (two ends and the middle) of each bag by the use of sampler or bamboo. According to the above-mentioned instruction, the samples that are regarded as the elementary samples were huddled and after they were mixed, one-cup (including about 200-10000 grains, depending on type and size) equal to 100 grams of that was selected and picked as the final sample for analyzing the existence of the pest. Moreover, another sample was provided to analyze the probable existence of pest eggs. This sample were placed in freezer bags and kept in laboratories in Plexiglas containers at 28 centigrade for one month to hatch the pest eggs if there is any.

**Segregation of pests from cereal and grains:** the following methods were used to segregate the pests from cereals and grains:

- Direct observation with naked eye
- The analysis of contaminated grains by microscope and binocular
- Berlese funnel method
- Floating method

The segregation of pests from grain and cereal samples was done by direct observation and observation by naked eye. The pest samples (live and dead) and the skinned-off cuticle among cereals and grains were segregated. Covering or winnowing of the grains helped the pests’ segregation in this method. Moreover, the contaminated grains were exactly observed by binocular and microscope and were opened or broken if there was a pest and the pest was brought out. After that, the segregation of the pests remained in the samples of cereals and grains were done through Berlese funnel method. This method is mostly used to segregate the storage tick, mite so that the samples were poured in the glass funnel in which there was a filter paper and through turning on a light source above the funnel the ticks moved in the opposite side of light, and they were directed to the container of alcohol. To segregate the existed pests inside the grains, floating method was used. Through this method, grains contaminated with pests that were active inside the grain and not observable, were floated on the water due to the lightweight and were recognized from other grains. Then the grains were opened and their pests were segregated.

**Recognizing arthropod pest species:** The pest species were segregated and identified. In order to do that the detailed description of species and the special detection key of storage pests in Iran and the world
such as Bageri Zonooz [18, 4] and Gorham’s [19] researches were used. It also should be mentioned that the contamination to pest refers to the observation of alive or dead pest especially (including all the biological processes such as larvae, pupae, and mature), insect shell (cover or skinned-off cuticle) and created damage in the product (including holes and created fractures in the grain as the result of feeding and spawning). To count the insect seeds inside the product, the samples inside the incubation were used. Of course, in order to identify the species of samples, matured insects were only used. Weighting and counting were done for the grains of all the cereals and beans studied to have the weight comparison among the contaminated products.

It should be mentioned here that regarding the military situation of storages and observance of security issues, the names of storages were mentioned from number 1 to 9, and the geographical situation, area, and capacity of them were not stated. The amount of cereals and grains in the warehouses, as well as time of entrance and existence and the amount of stop time in each storage, even numbers and weight of selected bags for sampling (through which it’s possible to estimate the volume of products in storages), and generally any information that could be abused was kept as secret. Therefore, among the limitations of this study one can refer to impossibility of comparison of storages in accordance with the volume of their products.

**Statistical analysis:**

Counting number of pests based on existence or absence of mature alive pest, mature dead pest, pest seed, skinned-off cuticle pest, and destroyed seeds as the result of pest activity in each 100 grams from each product were determined. The amount of contamination was reported as mean±standard deviation in each 100 grams of the sample. The statistical analysis of data was performed with the analysis of the assumptions that the data are normal and the variance is stable. However, regarding the very large scattering of data and lack of establishment of variance stability assumption and regarding the fact that the data were numerical and the assumption of data normality was not established by the use of Kolmogorov-Simonov test or when the data normality assumption was established, the results of Kruskal Wallis test were presented in the present research. Moreover, if the Kruskal Wallis was significant, Mann Whitney test was used to compare the groups two by two. The comparison of the amount of contamination in each warehouse was reported at the standard limit (the index of 5contaminated grains in each 100 grams) descriptively, and due to the low times of its test (4 times), no statistical tests were used in this case. To analyze the data SPSS 12 were used.

**Results**

The weight of each grain and the numbers of them in each 100 grams in the military unit warehouses are shown in Table 1.

<table>
<thead>
<tr>
<th>Types of grains</th>
<th>The weight of each grain (gram)</th>
<th>The weight of 100 grains (gram)</th>
<th>The number of grain in 100 grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>0.01</td>
<td>1</td>
<td>10000</td>
</tr>
<tr>
<td>Bean</td>
<td>0.5</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>Lentils</td>
<td>0.06</td>
<td>6</td>
<td>1666</td>
</tr>
<tr>
<td>Pea</td>
<td>0.3</td>
<td>30</td>
<td>333</td>
</tr>
<tr>
<td>Barley</td>
<td>0.04</td>
<td>4</td>
<td>2500</td>
</tr>
</tbody>
</table>

Generally, the existed grains and beans in the warehouses studied, are composed of 11 types of different products including types of bean, split peas, chickpeas, peas, lentils, beans, wheat, barley, and types of rice (Iranian, Pakistani, and Thai) in different shares. Totally in 9 warehouses studied, among 91 products (through mentioning repetitions, and counting the shares), the sampling was done regarding the variety of product and existent amount of that in the warehouse. In terms of diversity, storages 6 and 5 had the most amounts of grains and beans with 12 and 13 types of products, respectively, and storage 2 had the least amount of diversity with 5 types of products that had no relation with the amount of product in each warehouse, regardless the type of pest and product, storages 1, and 3 had the most amount of contamination with owning the highest amount of contaminated grains in each 100 grams in the whole product and had a significant difference with other warehouses in this respect (p<0.05). In storages, 2 and 9 no contamination to arthropod pest in grains and beans was observed (Table 2).

Among types of grains and beans studied, products such as split peas, peas, types of beans (pinto, red, white), and wheat were not contaminated in all the warehouses and the samplings. Among all the grains studied, barley grain was the most contaminated product, and its contamination was significantly more than other products (p<0.05). Totally, the mean of barley grain contamination in eight storages was about 3.2±1.1 in 100 grams. The most contamination of barley was in the first storage (13.5 in each 100 grams) (Table 2). However, the numbers of contamination cases in the warehouses, bean had the most amount of contamination, and this product was...
Frequency and diversity of pest arthropods in stored cereals in a military unit

Contaminated in 6 storages (there were beans in eight cases from 9 cases of storages studied). The mean of bean contamination in warehouses studied was 0.75±0.3 in each 100 grams. The contamination of Thai rice was more than other types of rice; about 0.3 in each 100 grams of rice; however, there was no significant difference with other types of rice (p>0.05).

Table 2- The amount of contamination of grains and beans in military units warehouses based in Tehran in 2007-2009 (the unmentioned products in the table, are free from contamination)

<table>
<thead>
<tr>
<th>The number of warehouse</th>
<th>The number of products in warehouse</th>
<th>Types of the contaminated products</th>
<th>The mean of the contaminated grains in each 100 grams in each product</th>
<th>The mean of the contaminated grains in each 100 grams in the whole product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>Barely 13.5±3.3</td>
<td>1.3±0.65</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>Barley 11.8±3.9</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>Pakistani rice 0.5±0.5</td>
<td></td>
<td>0.1±0.05</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Thai rice 2±0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>Bean 3.3±2.3</td>
<td>0.3±0.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>Thai rice 1±1</td>
<td></td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>Bean 0.5±0.2</td>
<td>0.05±0.03</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>Bean 0.8±0.5</td>
<td>0.08±0.05</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3- The species of separated pests from grains and beans existed in the military units' warehouses in 2007-2009

In addition, 4 types of arthropod pests were identified among the grains and beans products in the storages studied. All the species of pests identified were from the orders of Coleoptera, and from three families of Silvanidae, Tenebrionidae and Chrysomelidae. The most frequent of storage pests studied, was the *Tribolium castaneum* species that are related to the barley product pest. Other grains and beans owned their own special pests. *Oryzaephilus Mercator* specie was observed in all three types of Iranian, Pakistani, and Thai rice. Each of the products studied, was contaminated to one type of arthropod maximally (Table 3).

Discussion

The results achieved from the food storages showed that products such as split peas, peas, and beans (pinto, red, and white) and wheat were not contaminated in all the warehouses. Products such as lentil, rice, and bean were contaminated in some of the warehouses, but this amount was less than the standard level. The standard level was 5 contaminated grains in each 100 grams of grains and beans maximally [17]. This issue shows the accuracy of analysis and healthy observations in the entrance level of products to the warehouse; moreover, this issue shows the suitable maintenance of the product and efficiency of control methods of arthropods pests in the warehouses studied. Although, some of the products were safe and free from any pest during one year of sampling, there were 3 species of Coleoptera pests in some other products such as split pea, rice, and bean. If the situation of the warehouses was suitable for growing the pests even for a short time, the danger of contamination would increasingly be provided for these insect pests and storages mites [4].

In this study, the contaminated products were only contaminated to one species of arthropod pest. Although, most of the grains and beans have special
pests, in some cases and in severe contaminations as well as unsuitable maintenance condition of the product in the warehouse, they might get contaminated to one or some species of pests [5, 10]. Probably, in storages under the study, observance of health issues, and proper storage conditions, prevents the contamination of grains and beans by various pests. The studies performed on the issue of storage pest of wheat in some warehouses and grain elevator of Iran such as that of Tehran, showed the contamination of some of the wheat loads to 9 species of arthropod pests (5 species from the order of Coleoptera) [3]. Among these pests, *Tribolium castaneum* specie was observed; however, in the storages of this study, the wheat was free from this pest as well as other arthropods. Among the 4 species of arthropod pests identified in this study that were from the branch of Coleoptera, *Tribolium castaneum* specie were observed as the barley pest. This pest is known as one of the most important pests of storage grains in the entire world. Moreover, this pest has a strong history as the storage pests of food products and can damage to a wide variety of storage grains such as pea, bean, grain, and nuts (such as almond, and pistachio), and even flour, cacao, and dried fruits [21, 22]. Therefore, it is necessary to do a lot of observation to prevent the proliferation of this species in warehouses.

In the studies done in other countries on the issue of proliferation of pests in agricultural products especially cereals, different results have been reported. In an analysis in Brazil, it turned out to be that out of 125 samples of wheat, barley, and the products, except 2 samples of wheat, the others have been contaminated to one or some pests such as beetle and butterfly [13]. Other studies done in Brazil showed that about 10% of storage grains destroy as the result of arthropod pest’s activity annually. The most important specie of wheat pest in this country is Australian beetle of wheat [16] that is regarded as one of the most important pest of wheat in Iran as well [5, 6]. Moreover, the studies showed that more than methyl bromide and phostoxin that are used to control the arthropods in warehouses, essences made from some species of thyme plant (*Thymus persicus*) in Iran can be effective in controlling the species of *Tribolium castaneum* [23].

Among the samples studied, it turned out to be that the barley contamination was more than the standard level only in 2 warehouses (number 1, and 3). In the other word, although 16 cases (17.6%) out of 91 products studied in 9 warehouses were contaminated, the contamination was more than the standard level of Iran only in 2 cases (2.2%). Of course, the different ideas on the complications and losses resulted from storage pests and the acceptable level of that caused some differences between the standard of Iran with that of other countries. These differences depend on the economical situation of different societies and the amount of sensitivity to agricultural products of each country to the storages pests [5, 6]. In those countries that laid down contamination standard for humans’ food, the acceptable level of contamination was determined from 20-30 numbers in each 100 grams of the product. However, most of the countries (such as Russia, Bulgaria, Armenia, and Estonia) have reported 25 numbers in each 100 grams as the standard level. The range of acceptable contamination for the livestock in different countries to the human’s food is more dispersed and is variable from 25-100 numbers in each 100 grams [24]. In Iran, in the regulation no. 5925 of Standard Institution and Industrial Researches of Iran, a standard level is determined for some of the storage pests. If we regarded it as the index, the standard and acceptable level of pest in livestock and poultry’s food is equal to 25 contaminated grains in 100 grams and for human’s food, it is 5 grains in 100 grams [17]. Therefore, based on Iran National standard, the amount of contamination was more than the acceptable level in 2.2% of cereals studied in this research (12% of barley product). This issue can show the suitable maintenance of cereals and grains in most of the warehouses of military units under the study. Of course, it should be mentioned that in determining the standard amount of contamination of products, the economical losses are mostly considered [5]; however, less amounts of the standard level of products pests can bring up health problems as well. Analysis have shown that although storage pests cause economical losses and decrease of the quality of product by feeding from grains and destroying nutrients, basic elements, and their vitamins; however, in many cases they can cause significant health loss by contamination of stored grains to stool and larval cuticle and insect nymphs. As an example, the aggregation of (*Trogoderma granarium*) in wheat stored in wheat elevator makes the provided flour get contaminated by the long hairs of larvae of the insect and cause severe disorders in the users’ GI tract. Among the different types of rice studied, it turned out that the contamination of Thai rice was more than the other ones. Although, the amount of this contamination was less than the standard and acceptable level and can increase and arrive at a standard and acceptable level if the contamination condition is ok at long-term maintenance times in warehouses. Moreover, they can contaminate the other
types of rice. Regarding the fact that rice is the main food of Iranians, more observation should be done at the purchase or entrance time of them to the warehouse. The observation of imported rice is often done once at the entrance time of rice to the country. In addition, among the storage pests, contamination to crab louse is only regarded [4]. Regarding the susceptibility of rice to contamination by arthropod pests such as Oryzaephilus mercator that’s observed in imported and Iranian rice in this study, the screening of consumable rice is necessary regarding the contamination to this pest in different levels. Rice cultivation is usually done in hot and humid weather that’s a good condition for contamination of that to the arthropod pest [4, 25]. The most part of internal rice is cultivated in Northern cities the weather of which is regarded as a suitable environment for the proliferation of farm arthropods. Studies showed that the farms that are situated in humid areas have more susceptibility to the contamination with arthropod pests in comparison with other areas [25].

Grains imported to Iran including rice are subject of measuring for storage pests. The import of grains and beans that own alive pest and losses more than the standard level is rejected. Therefore, at the time of entrance of the product to the warehouse, the amount of contamination may be at the standard level, but long-term storage causes the proliferation of contamination and growth of storage pest [26, 27, 28]. Therefore, it should be noted that in spite of the observations and controls done by the related health units to enter those raw food products to the warehouses that are free from pests, the products might contaminate in warehouses. Moreover, the contamination may exist as the pest seed inside the seeds and turn into matured pest at the maintenance time [4, 24, 29].

Regular and close periodic samplings especially in warm seasons (spring and summer) based on recommended scientific methods, during the warehousing can aware the officials’ warehouses by the contamination of products to the storage pest, prevent the proliferation of pest or secondary contaminations in warehouses.

Regarding the findings of this study, although the extent and domain of grains and beans contamination to storage pests was low, and except 2 cases in other cases, this contamination was lower than the standard level. In order to prevent the health complications of these pests in users, it’s recommended to measure the amount of these pests before purchasing of grains and beans for humans’ uses (and not at the entrance time of the product to the warehouse). In measuring the amount of contamination that is usually done by naked eyes, non-experts, and non-scientific method, the alive pests or contaminated grains are only regarded as the index of measuring; however, in most of the cases, the pest seeds are inside the seeds and this issue can be determined by the entomologists’ diagnosis and through different methods at high accuracy.

Conclusion

In the studied warehouses, there were 4 species of arthropod pests including Oryzaephilus mercator, Tribolium castaneum, Bruchus lentis, Bruchus rufimanus in rice, barley, lentils and bean, respectively. Warehouses number 1 and 3 had the most amount of contamination. Among the types of studied grains, barley had the most contamination. The amount of contamination in the grains and beans was less than the standard level in the studied warehouses and the contamination of barely was more than the standard level in two of the warehouses. It is recommended to measure the amount of storage pests in grains and beans through scientific methods before purchasing the grains and beans (and not at the entrance time of the product to the warehouse) to prevent health complications.

Acknowledgement: Authors would like to thank the Military Health Research Center that provided the funds of this research. The valuable cooperation of the National Plant Protection Organization and the Department of Entomology, especially Dr. Keyhanian, and Dr. Rajabi are highly thanked for their valuable consultations. Moreover, authors thank the Health Management and their agencies in Military Affiliated Centers, officials, and the responsible and personnel of food warehouses of the Military Unit without the faithful assistance of whom conducting this study was impossible.

References