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Authors: Sukirno, Sukirno, Husain, Mureed, Siswantoro, Muhammad, Rasool, Khawaja Ghulam, Shaheen, Farid Asif, et al.

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Study on the loss of value of Khodari date fruit infested by almond moth (Lepidoptera: Pyralidae)

Sukirno Sukirno[1,2], Mureed Husain[1,], Muhammad Siswantoro[2], Khawaja Ghulam Rasool[1], Farid Asif Shaheen[3], Shehzad Salman[1], and Abdulrahman Saad Aldawood[1]*

Abstract

Postharvest almond moth (*Cadra cautella* Walker; Lepidoptera: Pyralidae) infestation is one of the primary challenges for production of dates, *Phoenix dactylifera* L. (Arecaceae) cv. 'Khodari.' This study was a simulation of early almond moth infestations in uncontrolled storage conditions. It aimed to investigate the effects of low level almond moth infestations on the population growth of the moth, the damage to the dates, bacterial and fungal contamination, and the nutritional value of the dates. One, 2, and 3 pairs of male and female moths were introduced into 250 g Khodari date samples. Noninfested dates were used as controls. Each treatment was performed in 10 replicates. The results showed a more than 25-fold increase in the almond moth population and significant damage to the dates. The moth population in the 2 pair treatment was the highest. Moth infestation also increased microbial contamination by more than 3-fold compared with noninfested dates. The treatment of the 3 pairs of moths significantly increased the bacterial load more than 20-fold when compared to the control. The fungal contamination in the 2 and 3 pair treatments were more than 5-fold higher than that of the control. In contrast, the moth pairs did not significantly affect the sugar, protein, fiber, ash, or water contents of dates. The results suggest that infestation by a single pair of almond moths at the initial stage postharvest causes significant damage and contamination in Khodari dates.

Key Words: dates; almond moths; *Cadra cautella*; microbial contamination; nutrition

Resumen

La infestación de la polilla de la almendra en poscosecha (*Cadra cautella* Walker; Lepidoptera: Pyralidae) es uno de los principales desafíos en la producción de los dátiles Khodari. Este estudio fue una simulación de infestaciones tempranas de la polilla de la almendra en condiciones de almacenamiento no controladas. Su objetivo era investigar los efectos de bajo niveles de infestación de la polilla de la almendra sobre el crecimiento de la población de la polilla, el daño a los dátiles, la contaminación por bacteria y hongo, y el valor nutricional de los dátiles. Se introdujeron 1, 2, y 3 pares de polillas macho y hembra en muestras de dátiles Khodari de 250 g. Se utilizaron dátiles no infestadas como controles. Se realizó 10 repeticiones de cada tratamiento. Los resultados mostraron un aumento de más de 25 veces en la población de polillas de almendras y daños significativos en los dátiles. La población de polillas en el tratamiento de 2 pares fue la más alta. La infestación de polillas también aumentó la contaminación microbiana en más de 3 veces en comparación con los dátiles no infestadas. El tratamiento de los 3 pares de polillas aumentó significativamente en más de 20 veces la carga bacteriana en comparación con el control. La contaminación por hongos en los tratamientos de 2 y 3 pares fue más de 5 veces mayor que la del control. Por el contrario, los pares de polillas no afectaron significativamente el contenido de azúcar, proteína, fibra, cenizas o agua de los dátiles. Los resultados sugieren que la infestación por un solo par de polillas de almendras en la etapa inicial posterior a la cosecha causa daños y contaminación significativos en los dátiles Khodari.

Palabras Clave: dátiles; polillas de almendras; *Cadra cautella*; contaminación microbiana; nutrición

The Kingdom of Saudi Arabia is the second largest global producer of high-quality dates. Its annual productivity estimates are as high as 1 million tons of date fruits, with a value of US $500 million (FAOSTAT 2014). Of this, almost 50,000 tons of surplus dates are exported annually (Al-Shreed et al. 2012). Approximately 400 date palm varieties are grown in Saudi Arabia (Al-Fuhaid et al. 2006) and dates are one of the country's major crops. Date fruits are well known for their high nutritional value. They contain free sugars (> 50%), dietary fiber (about 50%), proteins (6%), and minerals (Myhara et al. 1999; Al-Farsi et al. 2005; Al-Khalifah et al. 2013), as well as flavonoids, which are important as antioxidants (Hong et al. 2006).

Among hundreds of cultivated date palm varieties in the Kingdom of Saudi Arabia, Khodari date fruits, having a sweet taste, dark brown color, and dry texture, are among the most commonly exported date (Al-Shreed et al. 2012). They contain high levels of dietary fiber (60%), and the dried fruits have characteristic longitudinal fissures in their

[1]Economic Entomology Research Unit, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia; E-mail: sukirnobiougm@ugm.ac.id (S. S.), mbukhsh@ksu.edu.sa (M. H.), gkhawaja@ksu.edu.sa (K. G. R.), shehzadsalman@gmail.com (S. Sa.), aldawood@ksu.edu.sa (A. S. A.)
[2]Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; E-mail: sukirnobiougm@ugm.ac.id (S. S.), muhammad.siswantoro@gmail.com (M. S.)
[3]Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46300, Pakistan; E-mail: shaheen@uaar.edu.pk (F. A. S.)
*Corresponding author; Email: mbukhsh@ksu.edu.sa

skin (Al-Khalifah et al. 2013), and also contain high amounts of antioxidants and phenolic compounds (Al-Jasass et al. 2015). Fruits not fit for human consumption are mostly used for livestock feed (Vandepopuliere et al. 1995; El-Deek et al. 2010). This variety is well known also for its suitability for long-term storage when dried.

Under storage conditions, date fruits are vulnerable to the attack of several insect pests. There are 6 known insect species that infest stored dates, including almond moth (*Cadra cautella* Walker; Lepidoptera: Pyralidae), saw-toothed grain beetle (*Oryzaephilus surinamensis* (Linnaeus); Coleoptera: Silvanidae), dried fruit beetle (*Carpophilus hemipterus* (Linnaeus); Coleoptera: Nitidulidae), date stone beetle (*Coccotrypes dactyliperda* Fabricius; Coleoptera: Curculionidae), and rusty grain beetle (*Cryptolestes ferrugineus* Stephens; Coleoptera: Laemophloeidae). Among these insect pests, the almond moth is the most important pest of dried date fruits (Alzadjali et al. 2006). Insect infestations might cause reductions in weight, nutritional content, and commercial value, which also may lead to microbial contamination (Abu-Zinada & Ali 1982; Aidoo et al. 1996; Hamad et al. 2012; Aljasass et al. 2016). This may be hazardous to humans and inferiority may cause difficulties in the date commodity trade (Al Hussain & Jafar 1968).

Vulnerability to damage and losses caused by the almond moth have been reported for several products (Cox 1975; Hagstrum & Stanley 1979; Allotey & Goswami 1990; Ryne et al. 2006; El-Habbab et al. 2017). Almond moth populations also have been monitored in date orchards using pheromone traps (Ahmad 1987). Marouf et al. (2013) used 'Deyri,' 'Zahedi,' 'Piarom,' and 'Rabbi' date varieties and found that the Deyri variety was the most susceptible to almond moths. Almond moth also is a serious pest of stored maize in South Carolina, USA (Arbogast & Chini 2005). It also has the potential to be a stored insect pest of other dried fruits such figs, berries, and raisins (Marouf et al. 2011).

The almond moth begins to infest date fruits at the time of harvest from the field, although the infestation level is low. It is standard practice for conventional date growers to harvest and store dates without prior treatment to reduce pest population and in uncontrolled conditions, where pest population may continue to damage the fruit (S. Sukirno, personal observation). For example, in the Zulfi and Huraymila Districts (Riyadh Province) of the Kingdom of Saudi Arabia, most of the farmers store semi-dried date fruits directly without applying any treatments. This makes the stored dates vulnerable to increases in the almond moth population. However, limited information is available about the potential population growth of almond moths and the damage caused by low density infestations of almond moth in normal storage conditions in the Kingdom of Saudi Arabia. Our first objective was to evaluate the vulnerability of Khodari date fruit to low almond moth population densities. Our second objective was to determine the effects of almond moth infestation on date fruit quality, including their nutritional value, and the risks of bacterial and fungal contamination in simulated uncontrolled storage conditions.

Materials and Methods

ALMOND MOTH COLONY

Almond moths used in this experiment were obtained from an insect colony maintained on an artificial diet (Al-Azab 2007; Husain et al. 2015) at the Economic Entomology Research Unit, College of Food and Agriculture Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia. The insect colony was reared in a growth chamber (Steridium, Q Super Centre, Queensland, Australia) under conditions of 65 ± 5% RH, 16:8 h (L:D), and 25 ± 2 °C. Freshly laid eggs (approximately 500 eggs) were placed onto 250 g artificial diet surfaces inside a 1 L plastic box (160 mm × 100 mm × 70 mm) (Al-Rasheed Co., Riyadh, Saudi Arabia). The eggs were incubated on the artificial diet until the adult stage emerged. Under the given conditions, on average the moths took 40 d to complete the life cycle from egg to adult (Aldawood et al., 2013).

EXPERIMENTAL DESIGN AND LIFE HISTORY OF ALMOND MOTH

Before the experiment, dates bought from the local market were sorted, and uncracked and noninfested dates were used. Dates frozen at −20 °C for 2 wk to eliminate any possible insect infestations. Prior to the experiment (24 h), the dates were placed in plastic bags and thawed at room temperature (25 °C). Approximately 250 g Khodari dates were placed in a 1 L plastic box used for almond moth mass rearing. Then newly emerged virgin males and females collected from the mass insect rearing were introduced into the dates and incubated in the same manner as for the mass rearing. Treatments consisted of 1, 2, or 3 pairs of adults, performed in 10 replicates. Noninfested dates as the control were maintained in the same conditions. All replicates were prepared and infested at the same time using the same almond moth cohort.

The artificially infested dates were maintained in a growth chamber (Steridium, Q Super Centre, Queensland, Australia) under the conditions of 65 ± 5% RH, 16:8 h (L:D), and 25 ± 2 °C. The introduced adults were kept for their entire life span and observed daily. After the moths died, the insect's remains were removed from the dates and discarded. The dates were further incubated until a new generation of adult moths began to emerge. The number and sex of emerged adults were recorded. Adults were collected daily until 1 mo from the date on which adults had first emerged. After this, all the almond moth stages were collected and recorded. Dates were weighed and the lost weight was calculated, and the number of damaged date fruit was counted. Damaged fruits were characterized by the presence of cracks on the fruit skin, frass inside or outside of the fruit skin, larval webbing on the fruit surface, and signs of fungal or bacterial growth (i.e., hyphae or oozing substances on fruit surface).

EFFECTS OF ALMOND MOTH INFESTATION LEVELS ON FEMALE FECUNDITY

To determine if the density treatments affected the fecundity of new generation moths, 4 pairs of virgin male and female moths were randomly selected from each treatment and their progeny were counted. Four replicates consisting of 1 virgin male and 1 virgin female from each of the above treatments were used to evaluate fertility. Each moth pair was placed in a plastic cup (50 mm diam × 70 mm high) (Al-Rasheed Co., Riyadh, Saudi Arabia) with a perforated lid (5 holes, 1 mm each), and provided with cotton soaked in 10% sugar solution. The eggs laid from each treatment were recorded daily and incubated at 65% RH, 16:8 h (L:D), and 25 °C. The number of larvae that emerged were recorded and then discarded from the cup daily until the tenth day of incubation.

EFFECTS OF ALMOND MOTH INFESTATION LEVELS ON BACTERIAL AND FUNGAL CONTAMINATION LEVELS

A 90 mg date sample from each treatment was taken and homogenized in 8.1 mL sterilized ultrapure water (Direct-Q® Water Purification System, Merck Millipore, Darmstadt, Germany) using an autoclave-sterilized pestle. After that, the sample was vortexed for 2 min at 2,400 rpm. A 100 µL sample of the solution was poured into a sterilized Petri dish, and then 10 mL of potato dextrose agar (Scharlau Microbiology, Scharlab Inc., Barcelona, Spain) and nutrient agar (Merck Microbiology, Merck, Darmstadt, Germany) for fungal and bacterial contamination studies, respectively, was poured evenly into the Petri dish. The media were prepared following the protocol and samples were incubated at 25 °C. The number of colony-forming units was counted after 48 and 24 h for fungal and bacterial growth, respectively. Five replicates of each treatment were used for this study.

EFFECTS OF ALMOND MOTH INFESTATION ON DATE FRUIT NUTRIENT VALUES

The nutritional values of date fruits exposed to each density of almond moth were evaluated based on the contents of total sugars, total protein, fiber, water, and ash. Three replicates of each treatment were used for this study. For total sugar measurement, 5 g of date samples were dried at 150 °C for 5 h in the oven (Universal Oven UN110, Memmert GmbH Co., Schwabach, Germany). After drying, 2 g of each date powder sample was used for sugar content analysis by using the Nelson Somogyi method (Sudarmadji 1997). Sugar quantification was measured using a spectrophotometer (GENESYS™ 10S UV-Vis Spectrophotometer FUW220PA ADVANTEC, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 540 nm wavelength and glucose as a standard. The standard was made by diluting glucose for 2, 4, 6, 8, and 10 mg in a total volume of 100 mL.

Protein content of dates were analyzed using the Kjeldahl methods (Lestari et al 2018). Five hundred mg of each sample was used for this analysis. The catalyst and an indicator used were Na_2SO_4: HgO (20:1) and methyl red-methyl blue, respectively. Titration was conducted using 0.02 N of HCl. For the total fiber measurement, a date sample was ground using a commercial grinder (Philips HR 2115, Jakarta, Indonesia) then filtered using a sieve of 1 mm diam mesh. Two g of sample that passed through the sifting then was used for crude fiber quantification using the gravimetric method (Andarwulan et al. 2011), and the final residual weight was measured as crude fiber content. The moisture of the samples was measured using 2 g of dates. The date was weighed and placed into a bottle with a known mass, then dried and heated at 150 °C for 5 h in the oven (Universal Oven UN110, Memmert GmbH Co., Schwabach, Germany). Then the sample was chilled in the exsiccator (Sigma Aldrich, Burlington, Massachusetts, USA) and weighed. Subsequently, the sample was heated for 30 min, then chilled and weighed again. This procedure was repeated until the weight was constant (Lestari et al. 2018).

$$(\%\ \textit{wet basis}) = \frac{\textit{initial weight} - \textit{final weight}}{\textit{initial weight}} \times 100\%$$

Ash content of the date samples was measured using procedure described by Lestari et al. (2018). A 5 g sample of each treatment was weighed, then put in a porcelain crucible and heated in the electric stove until it turned into charcoal. After that, it was burned in the muffle furnace (Thermolyne, Taylor Scientific, St. Louis, Missouri, USA) until it turned into white ash. The ash was put into the oven at 100 °C, then removed to an exsiccator for chilling and weight. The procedure of burning in the muffle furnace was repeated until a constant weight was obtained (Lestari et al. 2018).

STATISTICAL ANALYSIS

The experiment was conducted in a randomized design, and analysis of variance (ANOVA) at α:0.05 was performed to compare almond moth populations and the effect of almond moth infestation levels on date damage, fungal and bacterial contamination, and date nutrient content. The parameters in percentages were transformed by using log prior to ANOVA to meet a normal distribution. Treatment means were compared by using Tukey's honestly significant difference test at α:0.05. The statistical procedures were done using SPSS vers. 13.0 (SPSS 2005).

Results

LIFE HISTORY TRAITS OF ALMOND MOTHS REARED ON KHODARI DATES

The total numbers of larval, pupal, and adult stages of almond moths from different initial populations are shown in Table 1. The results showed significant population growth. There were no significant differences between the total numbers of individuals in the larval stage ($F_{2,27}$ = 2.26; P = 0.12), the average numbers of female ($F_{2,27}$ = 0.43; P = 0.67), and male ($F_{2,27}$ = 2.86; P = 0.07). The average numbers of pupae ($F_{2,27}$ = 3.36; P = 0.05) and total population ($F_{2,27}$ = 4.75; P = 0.02) were significantly different.

The initial density of almond moths attacking date fruits did not affect the fertility or fecundity of new generation moths (number of eggs: $F_{2,9}$ = 0.44; P = 0.66; hatched larvae: $F_{2,9}$ = 0.49; P = 0.628 (Table 1). Each female produced an average of 185.25 to 300.75 eggs. These results indicated that low levels of almond moth infestation had more potential to produce higher levels of infestation in the next generation.

AFFECTS OF ALMOND MOTH INFESTATION LEVELS ON DAMAGE IN KHODARI DATES

Table 2 shows that a low levels of infestation damaged the dates with no significant differences in weight loss ($F_{2,27}$ = 2.71; P = 0.09) or weight loss percentage ($F_{2,27}$ = 2.71; P = 0.08). The total numbers of dates used for the experiment was not significantly different ($F_{2,27}$ = 1.77; P = 0.19). Each 250 g consisted of 20 to 22 date fruits. The almond

Table 1. The population densities (mean ± SE) of almond moth first generation on artificially infested Khodari dates with 1, 2, and 3 pairs of moths; numbers in each row followed by the same letter were not significantly different (P < 0.05).

	Number of moth pairs		
Parameters	1	2	3
Larvae	15.10 ± 3.86 a	37.90 ± 9.19 a	25.80 ± 8.56 a
Pupae	0.10 ± 0.1 a	0.30 ± 0.3 ab	1.40 ± 0.58 b
Male adults	31.1 ± 6.14 a	56.30 ± 5.78 a	60.80 ± 9.96 a
Female adults	32.30 ± 6.37 a	57.90 ± 8.15 a	62.60 ± 7.81 a
Total population	78.60 ± 14.85 a	152.40 ± 20.84 b	150.60 ± 18.65 b
Number of egg progeny (F2)	185.25 ± 70.55 a	300.75 ± 136.54 a	291.25 ± 72.86 a
Number of larvae (F2)	177.75 ± 72.6 a	292.50 ± 136.40 a	287.50 ± 71.32 a

Table 2. The damage level (mean ± SE) of Khodari dates exposed to 3 densities of adult almond moth; numbers in each row followed by the same letter were not significantly different (P < 0.05).

	Number of adult pairs		
Parameters	1	2	3
Weight loss (g)	58.83 ± 5.15 a	77.64 ± 3.91 a	81.32 ± 10.92 a
Weight loss (%)	23.53 ± 2.06 a	31.05 ± 1.56 a	32.53 ± 4.37 a
No. of dates before treated (*n*)	20.9 ± 0.23 a	21.5 ± 0.31 a	21.4 ± 0.16 a
No. consumable dates (*n*)	4.2 ± 2.8 a	0 ± 0 a	0 ± 0 a
No. unfit dates (*n*)	16.7 ± 2.71 a	21.5 ± 0.31 a	21.4 ± 0.16 a
Damage %	80.48 ± 13.02 a	100 ± 0.00 a	100.00 ± 0.00 a

moth infestations made date fruits unfit for human consumption. The number of damaged date fruits between treatments did not differ ($F_{2,27}$ = 2.25; *P* = 0.13). Infestation with 1 pair of moths caused 80.5% of the dates to be damaged. In treatments with 2 and 3 pairs of almond moths, 100% of the dates were unfit for human consumption at the end of the experiment.

EFFECTS OF DIFFERENT ALMOND MOTH INFESTATION LEVELS ON BACTERIAL AND FUNGAL CONTAMINATION OF KHODARI DATES

Levels of bacterial and fungal contamination of moth infestation on dates are depicted in Table 3. There was a significant difference in the bacterial contamination level ($F_{3,16}$ = 9.64; *P* = 0.007); it tended to be higher than in the control. The results indicated that the treatment (number of moth pairs) affected both bacterial and fungal contamination. This indicated that moth infestations increased bacterial contamination 4- to 21-fold compared to the control. Fungal contamination between treatments also was significantly different ($F_{3,16}$ = 6.36; *P* = 0.03). Fungal contamination was 3- to 7-fold higher in the infested treatments than the control. These data suggest that almond moth infestations may stimulate an increase in bacterial and fungal contaminations.

EFFECTS OF ALMOND MOTH INFESTATION ON DATE FRUIT NUTRIENT VALUES

The effects of almond moth infestation on the nutrient values of Khodari dates are shown in Table 4. For most of the nutritional values tested, there was no effect of almond moth treatments. The analysis showed that the infested dates, compared with the control, had a slightly higher water content ($F_{3,8}$ = 2.016; *P* = 0.19), ash ($F_{3,8}$ = 1.50; *P* = 0.287), protein ($F_{3,8}$ = 1.351; *P* = 0.325), and fiber ($F_{3,8}$ = 1.351; *P* = 0.325). For sugar content, there was a slight decrease in the almond moth infested treatments ($F_{3,8}$ = 1.192; *P* = 0.373).

Discussion

The primary problem in date production in Saudi Arabia is found at the postharvest level. During the postharvest period, insect infestations and microbial contaminations are significant. Studies showed 5% to 10% production loss in Khodari dates. Of these losses, 21% are caused by insects. Insect infestations decrease the quality and quantity of the date harvest. It is estimated that 12% of fruits at the market were infested by insects (El-Habbab et al. 2017).

A study of biological traits of the almond moth conducted by Husain et al. (2017a) showed that almond moths reared on Khodari dates had 6 larval instars. Their data also showed that, at 25 °C, each female laid 213 eggs and 85.9% of them hatched. However, in another study using an artificial diet, each female almond moth laid 422 eggs, of which 78.7% hatched (Aldawood et al. 2013). These averages of hatched eggs were lower than those found in our study. Out of 185 eggs, 177 (95.9%) hatched, and 287 out of 300 eggs (98.7%) hatched in the treatments consisting of 1 and 3 pairs of almond moths, respectively. These data indicate that date fruits have high nutritional values that support moth fecundity. In this study, the almond moth population increased by approximately 39-, 38-, and 25-fold in treatments with 1, 2, and 3 pairs of almond moths, respectively. These were higher than previous results found using peanuts, in which the almond moth population increased by 37-fold (Hagstrum & Stanley 1979). These results imply that even a low initial infestation of almond moths (1 pair) may pose a high risk of damage to the stored dates.

Based on our data, the damage levels caused by the initial moth infestation showed that 1 pair of almond moths resulted in significant losses in each of 250 g Khodari date samples. These data suggest that, although the initial infestation by the almond moth was low, it may cause significant damage to dates, and as such, attention should be paid to their management.

Almond moth infestations increased bacterial and fungal contamination at least by 4-fold compared to noninfested dates (Table 3). A study by Al Hazzani et al. (2014) reported that *Escherichia coli* (Migula) Castellani & Chalmers (Enterobacteriaceae) contamination was present in relatively high amounts in raw Khodari date fruits. This may be caused by the storage conditions of date fruits because they are not held under refrigeration, thus creating suitable conditions for the proliferation of microbes. Their study also found 14 species of fungi on Khodari dates. The *E. coli* was known to be a pathogenic bacterium (Hamad 2012). The presence of *Aspergillus niger* van Tieghem (Trichocomaceae), *Penicillium* sp. (Trichocomaceae), *Rhizopus* sp. (Rhizopodaceae), and *Fusarium* sp. (Nectriaceae) may stimulate the formation of

Table 3. The mean numbers of bacterial and fungal contamination levels (× 10^5 colony-forming units per g) of Khodari dates exposed to 3 densities of adult almond moth; numbers in each row followed by the same letter were not significantly different (P < 0.05).

		Number of adult pairs		
Parameters	Noninfested	1	2	3
Bacterial contamination	1.00 ± 0.26 a	4.15 ± 0.76 a	5.78 ± 0.89 a	21.28 ± 8.17 b
Fungal contamination	0.86 ± 0.33 a	2.89 ± 0.24 ab	6.11 ± 2.00 b	5.11 ± 1.13 b

Table 4. The nutritional value (mean ± SE) of Khodari dates exposed to 3 densities of adult almond moth; numbers in each row followed by the same letter were not significantly different (P < 0.05).

Parameter	Control	Number of adult pairs		
		1	2	3
Water (%)	23.65 ± 1.17 a	29.02 ± 0.15 a	28.46 ± 0.58 a	28.92 ± 0.52 a
Ash (%)	1.76 ± 0.13 a	2.08 ± 0.22 a	2.70 ± 0.64 a	2.23 ± 0.17 a
Protein (%)	1.90 ± 0.13 a	2.42 ± 0.25 a	3.08 ± 1.06 a	2.69 ± 0.26 a
Fiber (%)	1.30 ± 0.37 a	1.30 ± 0.26 a	1.80 ± 0.26 a	1.55 ± 0.21 a
Sugar (%)	57.35 ± 0.61 a	53.70 ± 3.25 a	52.15 ± 3.49 a	50.41 ± 4.66 a

mycotoxins (Ibrahim et al. 2013) in dates, which are toxic to humans. These results indicate that attention should be paid to raising awareness of almond moth infestation in dates to avoid the risk of a high rate of microbial contamination.

The treatment of dates prior to storage is an important process to avoid microbial contamination. The use of deionized or tap water for 5 min before consumption may decrease the amount of contaminants present on dates; washing the dates also helps remove the maximum number of microbes (Hamad & Aleid 2013). The use of cold temperature conditions during storage also may help decrease the number of microbes on dates (Hamad 2012). The use of carbon dioxide alone (Husain et al. 2017b) or in a nitrogen mixture (Rasool et al. 2017) are promising for controlling the moth population.

In conclusion, infestation with even just 1 pair of almond moths has the potential to result in great losses of Khodari dates. The nutritional values were not significantly affected by the almond moth infestations, but the damage level and microbial contamination made the infested fruits unfit for human consumption. Therefore, the use of atmospheric manipulations at the storage of postharvest Khodari dates should become a priority, even if almond moth infestation levels are low.

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