Pathogenic effects of *Beauveria bassiana* and *Bacillus thuringiensis* on the population dynamics of *Ephestia Kuehniella*

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Abstract

The most dominant pest of date stored in Khuzestan Province is *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). In this study, the effects of *Bacillus thuringiensis* Kurstaki bacteria and *Beauveria bassiana* pathogenic fungi on the population dynamics of *E. kuehniella* date stock pest were investigated. To fulfill this, insectariums were kept in vitro at an average temperature of 27 ± 5 °C and a RH of 40 ± 5% for a duration of 6 months, equivalent to the maximum permitted shelf life of date. For sampling from 3 heights in 3 rows, one hole was randomly selected and a sample equal to 20 g of date was taken. Three samples were taken from each insectarium. During 25-week storage period of date treated with *B. bassiana* and *Btk*, 6 outbreaks occurred in the population of *E. kuehniella*. The mechanism of the epidemic is in the way that, once the population has increased to a certain threshold, the incidence of the pathogenic epidemic will be mass-dependent, leading to a decreasing trend in the pest population. Therefore, population reduction was estimated to be at 6 to 32 times that of the control. The highest disease mortality rate occurred at the egg and larval developmental stages, so the pathogens caused the highest mortality before the population entered the reproductive stage. Gradually, from the egg growth stage to the complete insect stage, a reduction was observed in the number of individuals which entered the subsequent growth stage. At all stages, the reduction in the treatment population group was faster than the control population. The highest reduction in the number of individuals entering the next developmental stage was associated with the larval developmental stage, followed by developmental stages of the adult, pupal, and egg. Regarding the highest potential of *B. bassiana* and *BtK* in reducing the stock pest population, it is very possible to exploit this interaction for biocontrol.

Keywords: Date palm, Mill moth, Entomopathogens, *Ephestia kuehniella*, Pathogenic effects

Background

The production of date in Iran is more than one million two hundred thousand kilograms per year. Accordingly, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is one of the most dominant stock pests of date palm in Khuzestan Province (Shakarami et al. 2015). The pest attacks stocked and stored date at the foot of the tree. Global attention is focused on minimizing spraying and control

costs, which may be achieved by using low-cost biologically effective insecticides (Rehab et al. 2020). Microbial pesticides have been proposed as environmentally friendly insecticides due to their short half-life, high degradability, and selectivity over certain species, which is an advantage for pest control (Arthurs and Dara 2018). On the other hand, in developing countries such as Brazil (Mascarin et al. 2019) and India (Kumar et al. 2019), the biological pesticides are a growing in the market; the biological pesticide are still under the same regulation as chemical pesticides (Arora et al. 2020).

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Various studies have been conducted on biological control of pests including fungal compounds (Latifian et al. 2018), bacterial and fungal combination (Mishra et al. 2013), and bacterial combination (Latifian and Kajbafvala 2015). Different strains of *Bt* bacteria and pathogenic fungi of insects have different synergistic effects on the pest control (Navon 2000 and Wraight and Ramos 2005). Recent trends in support of biological pesticides include the use of microbial agent mixtures (Sharifzadeh et al. 2018). This approach can lead to the prevention or delay in the development of insect resistance (Sudo et al. 2017), especially when the individual components of the preparation have different modes of action (Zhu et al. 2016). This strategy reduces costs (Das 2014) and preparation concentrations because microbial agent increases each other's activity and act synergistically (Wright and Ramos 2005). The two factors of host population density and pathogens have interactions and similarities in understanding the epidemiology of a disease among the insect population. In general, the population density of the pathogen is a mass-dependent factor (Gaugler and Kaya 1990).

Other research was conducted to evaluate the efficiency suitable Iranian isolate of B. bassiana for the microbial control of the flour moth on date. Results as the first step of feasibility of fungus application in microbial control showed that it had high potential for application on control of flour moth in date (Bahmani et al. 2012). The LC_{50} of *BtK* on *Batrachedra amydraula*, *E. kuehniella*, and *Plodia interpunctella* were 2.15×10^8, 5.18×10^8, and 2.71×10^8 CFU/ml, respectively (Latifian and Kajbafvala 2015). The lethal concentration, compatibility, and interaction effects of *Bt* and *B. bassiana* on *E. kuehniella* had been studied under laboratory conditions. Spores of *B. bassiana* at all concentrations showed a good computability with *Bt*. On the other hand, with increasing concentration of *Bt*, the amount of compatibility index decreased. *Bt* had synergistic effects in all treatments but the highest synergistic effect was observed in LC_{50} *B. bassiana* + LC_{50} *Bt* mixing on moth larvae. Given the highest compatible potential of *B. bassiana* and *BtK*, the possibility of exploiting this synergy is highly efficient to produce biocontrol (Bahmani et al. 2020).

In this study, the combined effects of *Bt* bacteria and pathogenic fungus *B. bassiana* on the population dynamics of *E. kuehniella* were investigated under the date storage conditions.

Materials and methods
Proliferation of fungal agent
The fungal isolate used in this study was obtained from research centers of date and tropical fruits of Iran. The characteristics of the studied isolate were IRAN 441C,

which collected in Systan and Balochestan Province (Saravan) of Iran.

The pathogenic fungus was scraped by transfer needle after performing complete sporulation of the culture medium surface (12–14-day culture) (Johnson et al. 1992). The spores were collected in separate Erlenmeyer flasks containing 10 cc of sterile distilled water with 0.05% Tween 80 solution. The abovementioned suspension was stirred in a pendulum for 5 min to uniformly distribute the spores. SDA + Y culture medium was used to increase the spore production (Thomas et al. 1987).

Preparation of *B. thuringiensis kurstaki* inoculum
Bacillus thuringiensis kurstaki (*BtK*) was used in this study. A pre-culture flask containing 500 ml of medium was incubated at 30 °C on an orbital shaker for 9 h and used to inoculate each of culture flasks. The composition of the pre-culture medium was as follows: glucose (10 g/l), ammonium sulfate (1.5 g/l), yeast extract (2 g/l), K2HPO4 (1.5 g/l), KH2PO4 (1.5 g/l), CaCl2 (60 mg/l), MgSO4 (500 mg/l), and MnSO4 (50 mg/l). The medium pH was adjusted to 7.0 by NaOH and autoclaved at 120 °C for 10 min (Azzou et al. 2015).

Preparation of fungal and *B. thuringiensis* formulation
Compatible formulation was produced at the production Strain Iran 441C was formulated as an oil-based emulsionable suspension (sesame oil) containing 3.49×10^3 spores/ml. All preparations were stored at 4 °C and retained high initial viability (> 93%) for the duration of the study. *Bt*, formulated as a flowable concentrate (3.49×10^3 CFU/ml), was produced by Abbott Laboratories, Inc. of North Chicago, Illinois, and stored at room temperature until use. Viability of conidia was determined by direct observation of conidia (× 400 magnification) plated on agar containing yeast extract (0.5%) and incubated 16–18 h at 25 °C. All conidia with visible germ tubes of any length were scored as viable (Wraight and Ramos 2005).

Reproduction of *E. kuehniella*
The flattened cap glass containers were used for reproduction of *E. kuehniella* in the laboratory. The container was heated to ensure that it was not infected. Every week, the female insects were transferred to new glass containers for laying. First, a growth medium was prepared for growing this moth. For this purpose, 1.5 kg of wheat flour plus 0.5 kg of bran were mixed with a small amount of yeast. After mixing, 1 g of moth eggs was poured on it and then covered with a clean cloth on a pan. By passing about 40 days, the first moths were observed and catching was begun. There were approximately 500 individuals of *E. kuehniella* inside each container, covered with a fine mesh cloth on the top of

each container. A funnel or cylindrical container was placed in a short distance from the bottom of the sheets until the moths laid their eggs from the lattice holes on the bottom of the container on the sheet.

Investigation of the pathogenic agents on the population dynamics

The glass insectariums, which resembled the date stock in storage, were used for performing the experiments. These glass insectariums were $100 \times 5 \times 5$ cm. On the one side of the vertical surfaces, 9 holes were created in 3 different heights rows at 25-cm intervals and were completely blocked by a special cap. Inside each of the insectariums was filled with 10 kg of Sayer date, about 500 dates each one weighing 20 g. For the 6 insectarium experiments, three containers containing the best fungal and bacterial combination treated date with 50% lethal concentration were considered, and the other three were considered as uninfected (as controls). Within each one of the insectariums, 250 full-sized *E. kuehniella* were transferred as the initial population. The insectariums were kept in vitro at the average temperature of $27 \pm 5\,^{\circ}$C and relative moisture of $40 \pm 5\%$ for a duration of 6 months, which was the maximum permitted shelf life of date stored. The temperature was adjusted by adjustable cooling and heating system at the constant temperature and moisture recording using a digital thermometer in the laboratory. The insectariums were sampled once a week.

Sampling of the pest population within insectariums

For sampling of each height, a hole was randomly selected from each row and a sample equal to 20 g of dates was taken. Three samples were taken from each one of the insectariums. The taken samples included different stages of pest growth. The following funnel was used to accurately evaluate the density of different growth stages of pests within the samples. For this purpose, 20 g of the sampled date from each one of the insectariums (including fungi and control containers) were individually transferred into a 250-ml Erlenmeyer flask, and then 20 g of sodium chloride, 30 ml of caustic soda, and 40 ml of water were added to it. Five milliliters of methylene blue was also added to the resulting mixture for staining. After that, the resulting mixture was transferred to a heat stirring machine until the mixture was boiling. After 2 min of boiling, the mixture was passed through a filtrate to separate the date pulps.

Various stages of pest development were transferred to New Erlenmeyer flasks with smoothing. Forty milliliters of odorless oil was added to Erlenmeyer flasks. Then, by the use of the separator funnel of this interstitial phase that included the growth stages of the pest population, different growth stages were counted by stereomicroscope. While studying the seasonal changes in different

stages of population growth using age frequency data analysis method, different pest growth parameters under the treatment and control conditions were compared to determine the effect of the combined treatment on population dynamics.

Data analysis

The age frequency data were obtained from sampling in terms of the abovementioned method at different times from the number of *E. kuehniella* at different stages of growth (q stage or age). These data were used to calculate population parameters such as survival rate and period duration of each stage (Wootton and Bell 1992; Wisdom and Mills 1997) so that the sum of individuals entering each age stage, mean time elapsed between age 1 to q-1, probability of survival from age 1 to q-1 (survival rate of each stage), mean time of entry to each stage, and the rate of survival time were estimated. The interval between the 2 samplings was 6 months of constant sampling and 1 week. Therefore, we have the following:

$$\hat{A} = \frac{1}{2} \sum_{1=1}^{n} (h_i + h_{i+1}) F_{ij} \qquad (1)$$

where Eq. (1), F_{ij} is the number of individuals at the age of Ith afterwards at time j. h_i and h_{i+1} are the sampling intervals between the time i and the next time (Wootton and Bell 1992 and Wisdom and Mills 1997). q is the number of growth stages, $f_j(t)$ is number of population at stage j for part of the population sampled at time t. $F_j(t)$ is the number at growth stages j, $j + 1$, q... for part of the population sampled at time t. M_j is the number that entered the growth stage j for part of the population sampled at time t. $g_j(x)$ is the function of density probability entering the stage j, μ is mean time of entering stage j, $e^{-\theta}$ is the probability of survival, for one time unit, a_j is growth stage duration j, w_j is the survival rate, specific for age j, A_j is the area under the age frequency curve of growth stage j, A_{*j} is the area under the curve for growth stages j, $j + 1$, q ..., D_j is the area under $tf_j(t)$ curve, and D_{*j} is the area under $tF_j(t)$ curve. D_j depends on average time to enter the growth stage j, the survival parameter, θ, and the area under the age frequency curve. These equations can be applied to age frequency cumulative data as follows (Wootton and Bell 1992 and Wisdom and Mills 1997):

$$A*_j = M_j\theta \qquad (2)$$

$$D_{j*} = \left(\mu_j + \frac{1}{\theta}\right)A_{j*} \qquad (3)$$

Using the two above equations, the survival rate of each growth stage can be calculated as follows. Here j is from 1 to q-1.

$$w_j = \frac{A*_{j+1}}{A*_j} \qquad (4)$$

The survival parameter was as follows:

$$\theta = - \ln\left(\frac{\frac{A*_q}{A*_1}}{\frac{D*_q}{A*_q} - \frac{D*_1}{A*_1}}\right) \qquad (5)$$

The stage j period will be as follows. Where the value of j varies from 1 to q-1:

$$a_j = - \frac{1}{\theta} \ln(w_j) \qquad (6)$$

And the number that entered the stage j (from 1 to q):

$$M_j = A*_j \theta \qquad (7)$$

When the sampling interval is equal, estimating the number between two times i and $i + 1$ enters the stage i and lived up to $i + 1$ can be calculated by the following formula (Wootton and Bell 1992 and Wisdom and Mills 1997).

Results and discussion

The weekly changes in the population density at different growth stages of *E. kuehniella* eggs, larvae, pupae, and moths in the two control and treatment populations (*B. bassiana* + *BtK*) under the storage conditions for a duration of 6 months were examined to be ensure that the results are presented in this topic.

Growth stage of the pest egg

There were two periods of activity in the population density change curve at this growth stage. The first period started from the 1st week and continued up to the 15th week. The peak of the egg density occurred around the 11th week. The second period started from the 16th week and continued up to the 24th week. The peak of the egg density during this period lasted about 22 weeks. A significant difference was found between the treatment and control conditions ($p = 0.000036$, $z = 4.13$, and df = 23). The egg density during the 25th week storage period was always significantly lower than the control conditions. Therefore, the maximum reduction was equal to 5.25 (Fig. 1).

The larval developmental stage

According to the curve shown in Fig. 2, during the 25-week storage period, there were 2 periods of activities in the larval population density change curve. The first period started from the 1st week and continued up to the week 11. The larval population density peak during this period was at week 6.
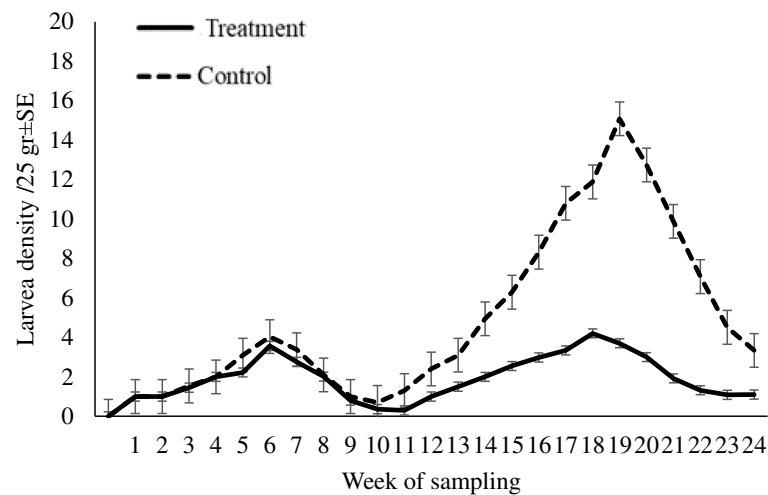
The second period started from the 12th week and continued up to the 24th week, and accordingly, the peak of larval population density was during the 20th week. According to these results, a significant difference was found between the treatment and control conditions ($p = 0.000013$, $z = 4.36$, and df = 23). The larval stage population density was always significantly lower in the treated date mass during the 25-week storage period compared to the control so that the maximum reduction was 5.36 times.

Pupal developmental stage

During the 25-week storage period, 2 periods of activities in the pupal population density change curve. The first period started from the 2nd week and continued up to the 13th week. The peak pupal population density was recorded at about the 8th week. The second period
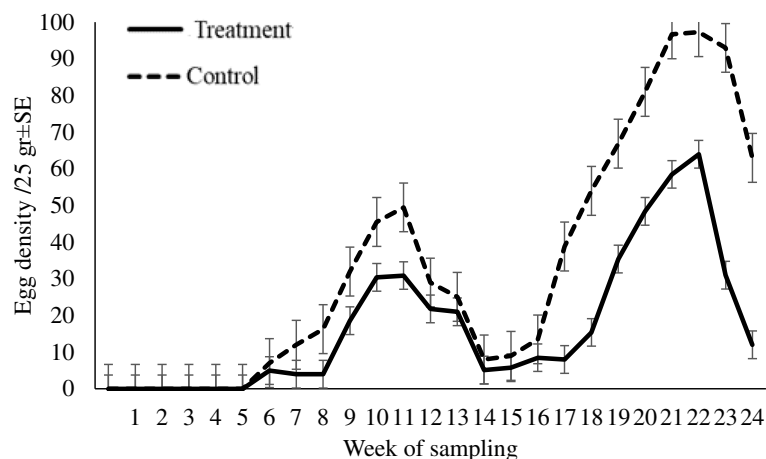
Fig. 1 The population density change curve of *Ephestia kuehniella* egg during 25 weeks of storage

Fig. 2 The population density change curve of *Ephestia kuehniella* larvae during 25 weeks of storage

started from the 14th week and continued up to the 24th week (Fig. 3).

The peak population density of this period was recorded around the 21st week. According to these results, a significant difference was found between the treatment and control conditions ($p = 0.00004$, $z = 4.57$, and df = 23). During the 25-week storage period, the pupal population density of the pest was always significantly lower than the control conditions. Therefore, the maximum reduction was 4.2 times.

Adult stage of insect growth

During the 25-week storage, 2 periods of activities in the adult insect population density were change the curve. The first period started from the 4th week and continued up to the 15th week, and the peak was recorded at

the 11th week. The second period started from the 16th week and continued up to the 24th week (Fig. 4).

Regarding, its peak occurred around the 22nd week. According to these results, a significant difference was found between the treatment and control conditions ($p = 0.00002$, $z = 4.25$, and df = 23). The population density of the adult insect was always significantly lower in the date treated during the 25-week storage period compared to the control so that the maximum reduction was 4.78 times.

Analysis of age frequency data in the treatment and control populations

The parameters of age frequency data for active population on the other cultivar treated with *B. bassiana* + *BtK* and the control were fitted as shown in Table 1.
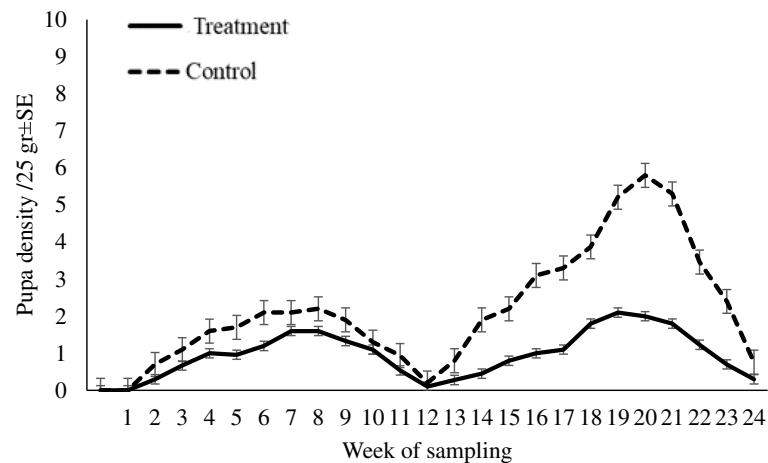
Fig. 3 The population density change curve of *Ephestia kuehniella* pupae during 25 weeks of storage
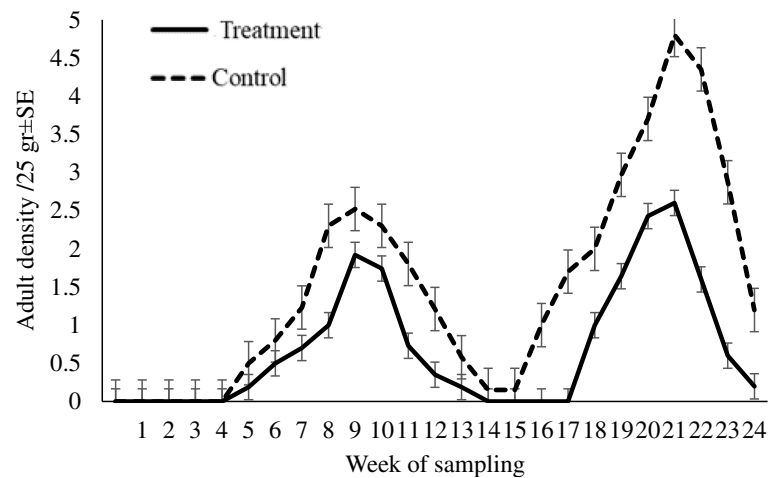
Fig. 4 The population density change curve of *Ephestia kuehniella* adults during 25 weeks of storage

In this table, M_j is the number sampled that entered the growth stage j for part of the population at time t. a_j is the growth stage duration j, w_j is the age-specific survival rate j, b_j is the number between two periods i and 1 i + entered the stage i, D_j is the area under $tf_j(t)$ curve, and A_j is the area under the curve at the growth stage j.

In Table 1, the parameters A_j and D_j are the area of the age frequency curve and the area under $tf_j(t)$ curve, respectively, which are the keys to estimate the other parameters. w_j is the population survival rate between the two growth stages. Comparison of this rate in the treatment and control populations indicated that the survival or transfer rate of the population from one growth stage to another stage during all stages except the larval stage in the treatment population is lower than the control population. In both of the populations, the highest mortality rate occurred from larval to pupal stages. In other words, the highest mortality occurred during the larval stage. Then, the highest mortality rate occurred during egg growing to the larvae. In other words, mortality at the egg stage was less important. This date was used to calculate the changes in populations treated with *B. bassiana* + *BtK* for different growth stages. The highest

survival rate was observed at the egg stage, and then at the larval and pupal stages, respectively (Fig. 5).
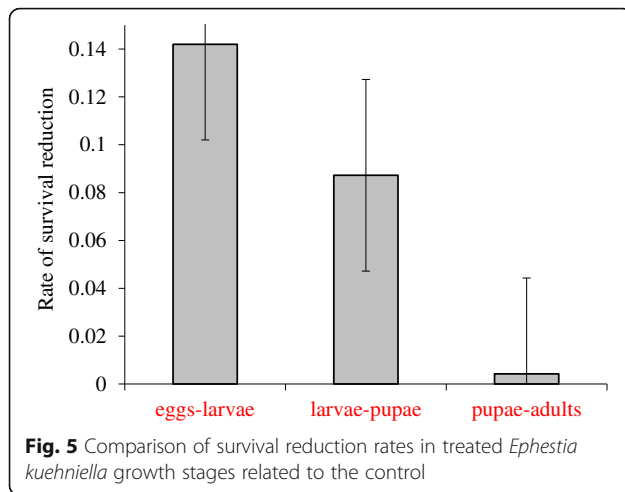
The parameter a_j in Table 1 shows the duration of the corresponding growth stage. The highest growth period was at the egg stage. Then, there were pupal and larval growth stages, respectively. The disease has caused a sharp reduction in the life span of all the growth stages. The highest reduction occurred at larval, pupal, and egg stages, respectively (Fig. 6).

The parameter M_j indicates the number of individuals entered the next growth stage. As shown in Table 1, a gradual reduction was observed in the number of individuals entered the growth stage from the egg growth stage to the adults. At all stages, the reduction in the treatment population was faster than the control population. The population reduction during different growth stages regarding the treatment with *B. bassiana* + *BtK* was estimated (Fig. 7). According to this figure, the largest reduction in the number of individuals entering the next growth stage was related to the larval growth stage, followed by the adult, pupal, and egg growth stages, respectively.

Results as an application can be programmed through the release of a combination of *B. bassiana* + *BtK* for
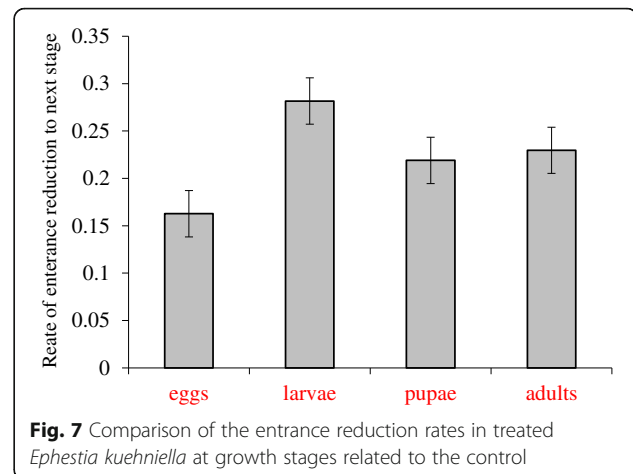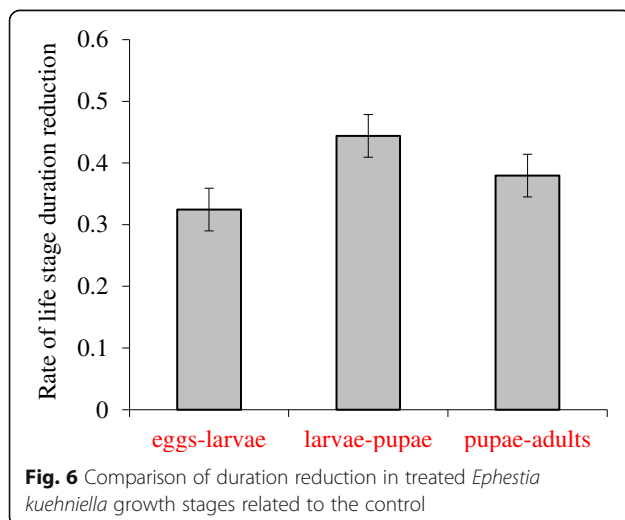
Table 1 Age frequency data parameters for the *Ephestia kuehniella* treatment and control populations

Populations	Life stage	Aj	Dj	Bj	Wj	Aj	Mj
Treatment	Egg	508.96	8484.3	16.7	0.1686	0.717	1250.5
	Larvae	87.16	1206.2	13.8	0.4686	0.305	210.87
	Pupa	40.96	593.7	14.5	0.4181	0.351	98.78
	Adult	17.2	268.5	15.6			41.29
Control	Egg	1018.7	17,657.6	17.3	0.196	1.062	1493.5
	Larvae	212.55	3385.55	15.92	0.431	0.5493	293.41
	Pupa	92.7	1443.25	15.67	0.420	0.566	126.48
	Adult	39.5	626.55	15.8			53.11

Fig. 5 Comparison of survival reduction rates in treated *Ephestia kuehniella* growth stages related to the control

Fig. 7 Comparison of the entrance reduction rates in treated *Ephestia kuehniella* at growth stages related to the control

Fig. 6 Comparison of duration reduction in treated *Ephestia kuehniella* growth stages related to the control

the management of date storage pests. The population change studies showed that the combined treatment due to high mortality rate at the larval stage and preventing the emergence of adult is effective on the management of stored date pests. In addition, the functional combination of *B. bassiana* + *BtK* suspension can result in better control of *E. kuehniella* compared to separately applying them. *B. bassiana* alone was not very effective on controlling the larval populations relative to other growth stages (Wraight and Ramos 2005). The time of population exposure to the control factor is also important. The results of the studies performed by other researchers suggested that repeated usage will be more effective under the conditions of high larval density (Johnson and Goettel 1993 and Inglis et al. 1996). In this study, a reduction of 5.36 times was observed at the growth stage of *E. kuehniella* larvae.

Of course, it should be noted that the control potential of *B. bassiana* is limited at high temperatures (Ekesi et al. 1999). Therefore, the effectiveness of *B. bassiana* can be increased by creating combined formulations such as *BtK* in application. The highest survival of *B. bassiana* in date mass also allows the pests to be exposed to fungal spores more frequently than the bacteria.

There are several reports of the correlations between *BtK* and *B. bassiana*. For example, the use of *Bt* of maize (*Zea mays*) increases the sensitivity of European maize beetles to *B. bassiana*. Also, the correlations between *B. bassiana* strain GHA and *Bt. tenebrionis* have been reported in the larval populations of Colorado potato beetles (Wraight and Ramos 2005). Several mechanisms have been proposed for the synergistic effects of *B. bassiana* and *Bt*. Some researchers have reported synergistic effects as a result of prolonging the time interval between molt due to endotoxin-induced toxins caused by *Bt* activity, thereby giving *B. bassiana* more time to penetrate the body cuticle before being eliminated (Wraight and Ramos 2005). On the one hand, larvae fed with food infected with *B. bassiana* spores acquired bacterial infection faster through the intestine (Ma et al. 2008) and on the other hand, starvation has been reported to increase the sensitivity to *B. bassiana* spores, and *BtK* disrupts the gastrointestinal tract in this way, spreading the epidemic in the host population infected with the fungus (Miranpuri and Khachatourians 1991).

The infection caused by the pathogenic activity of *B. bassiana* increases along with increase of the period between larval molt and reduction of intestinal cuticle excretion due to bacterial activity in the intestine. This mechanism may also explain the increased effect of *Bt* + *B bassiana*. Endotoxin of bacterial activity in the early larvae provides the background for most pathogenic fungi to enter the gastrointestinal tract, by passing a few days from the bacterial entry and a synergistic background for the complete onset of pest disease and death before entering the pupae (Furlong and Groden 2001).

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The results of the present study obtained a pesticide based on the synergistic yield of *BtK* and *B. bassiana*. Although other researchers have achieved similar results, there are different variables in each system that can have different effects on the final efficiency. Such differences have been observed in other researchers' studies (Costa et al. 2001). However, another important point is that, in addition to the isolates of *B. bassiana* and *Bt*, the constituents of modern formulations for the production of biological pesticides should also be compatible with the pathogenic activity of the two species (Vandenberg et al. 1998).

The surfactants can also interfere with and enhance or weaken the insecticidal activities of *B. bassiana* and *Bt* (Morris et al. 1995). The surfactants can affect the intestinal epithelium of insects and increase its permeability to bacterial toxins. The use of moisturizing and emulsifying agents in the formulation of *B. bassiana* and *BtK* aggravates the incidence of disease in target pests.

There are successful examples of the practical applications of *B. bassiana* and *BtK* interactions for the pest management system. For example, for having a full control of potato Colorado beetles over a 6-year period, it has been shown that the resulting biological pesticides, while lower costs than conventional management practices, can be used as a method for controlling the key elements of a combined multiple pest management system for the management of potato Colorado beetles. Clearly, such biological control programs have the potential to reduce the selective pesticide resistance strains (Cloutier and Jean 1998).

Regarding the high adaptive potential of *B. bassiana* and *BtK*, it is possible to exploit this coexistence for producing highly efficient and cost-effective biological pesticides. The microbial pesticides are costly and any strategy that allows a reduction in concentration makes their application in combined pest management more attractive. *B. bassiana* is mostly affected by environmental factors and its duration is longer, while *BtK* has a faster effect and a shorter duration. Therefore, the combination of these two can provide an appropriate control in many cases for pest larvae.

In general, the results of in vitro studies, which are the first step in the feasibility study of the use of *B. bassiana* and *BtK* in microbial combined control of *E. kuehniella*, showed that the combination of *B. bassiana* and *BtK* is of high epidemiological potential. Also, further studies are needed to find the suitable formulations for the combination of *B. bassiana* and *BtK* using vegetable oils and adding synergistic factors such as diatomaceous soil to its formulation because international experience has shown that vegetable oils are effective on enhancing the efficiency of formulations of insect pathogens. The diatomaceous soil also helps the host insect body to penetrate the fungus more rapidly and effectively by scratching the surface of the host body (Bell 1996). It is also necessary to investigate the effects of the combination of *B. bassiana* and *BtK* compared to other physical and chemical control methods on developing the control management plans.

Conclusion
B. bassiana and *Bt* had a high adaptive potential different growth stages of *E. kuehniella.* It is possible to exploit this coexistence for producing highly efficient and cost-effective biological pesticides for date palm stored pests.

Abbreviations
SDA + Y: Sabouraud dexterose agar + yeast extract; 1 to q-1: Survival rate of each stage; q: Stage or age; F_{ij}: Number of individuals at the age of Ith afterwards at time j; h_i and h_{i+1}: Sampling intervals between the time i and the next time; q: Number of growth stages; $f_j(t)$: Number of population of stage j for part of the population sampled at time t; $F_j(t)$: Number at growth stages j, $j + 1$, $q...$ for part of the population sampled at time t; M_j: Number that entered the growth stage j for part of the population sampled at time t; $g_j(x)$: Function of density probability entering the stage j; μ: Mean time of entering stage j; $e^{-\theta}$: Probability of survival, for one time unit; a_j: Growth stage duration j; w_j: Survival rate, specific for age j; A_j: Area under the age frequency curve of growth stage j; $A*_j$: Area under the curve for growth stages j, $j + 1$, $q ...$; D_j: Area under$tf_j(t)$curve; $D*_j$: Area under$tF_j(t)$curve; df: Degree freedom; z: z test; p: p value; M_j: Number sampled that entered the growth stage j for part of the population at time t; a_j: Growth stage duration j; w_j: Age-specific survival rate j; b_j: Number between two periods i and $1 i +$ entered the stage i; D_j: Area under$tf_j(t)$curve; A_j: Area under the curve at the growth stage j

Authors' contributions
All authors read and approved the final manuscript. Negar Bahmani: carried out the pathological and population studies and help to drafting the manuscript. Masoud Latifian: epizootiological studies, culturing of entomopathogens' data analysis, and drafting the manuscription. Hadi Ostovan: help to drafting manuscript and data analysis. Shahram Hesami: help to dafting the manuscription and experiments.

Availability of data and materials
All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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