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Adaptation and establishment of *Habrobracon hebetor* Say in the population of stored moths pests of date, *Ephestia kuehniella* Zeller and *Plodia interpunctella* Hübner

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Abstract

Background *Habrobracon hebetor* (Hymenoptera: Braconidae) is a larval ectoparasitoid, mainly of pyralid moths of the stored pests of date, *Plodia interpunctella* Hübner and *Ephestia kuehniella* Zeller. It has some suitable characteristics for use in biological control of stored moth pests. The aim of this study was to compare the ability of *H. hebetor* establishment in the population of two important date storage moth pests *P. interpunctella* and *E. kuehniella*, and its role in their population fluctuations.

Results The population growth trends of two host species increased in the first 6 weeks and in the 17th to 22nd weeks of the 6-month period of conducting tests. The peaks of larval population of the two species were in the 10th and 21st weeks under temperature of 25 ± 2 °C, RH of 60 ± 5% and a photoperiod of 12:12, D:L. The pattern of fluctuation in the parasitism percentage in the larval population of the two moth species host was inconsistent with each other. The parasitism percentage was higher on the larval population of *E. kuehniella* than larval population of *P. interpunctella*. In *E. kuehniella*, the host population change rates were − 0.1 in the 10th and 15th weeks, while the highest rates were 0.1 and 0.8 in the 12th and 3rd weeks. The lowest level of parasitoid balance in the larval population of *E. kuehniella* and *P. interpunctella* was recorded in the 11th and 15th weeks, and the highest levels were recorded in the 20th and 24th weeks, respectively. The maximum probability rate of parasitoid non-establishment in *E. kuehniella* larval population was zero during the entire period and for *P. interpunctella* until the 22nd week.

Conclusion In fact, there was a strong correlation between establishment success and the severity of host and parasitoid populations. Only in the larval population of *P. interpunctella*, the maximum probability of failure of establishment was 11.5%, due to the occurrence of the maximum population of parasitized larvae and the minimum ratio of non-parasitic and parasitic larvae.

Keywords Date palm, Indian moth, Dry fruit moth, Biological control, *Habrobracon hebetor*

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Background

Habrobracon hebetor is an ectoparasitoid of the larval stage of the Indian moth *Plodia interpunctella* and the dried fruit moth *Ephestia kuehniella* (Maafi and Chi 2006). Many biotic and abiotic factors such as temperature, humidity, females' age, host species, host size, and diet are influential on this parasitoid wasp efficiency (Gürbüz and Aksoylar 2006). The host species is influential in the parasitoid's searching behavior and its population dynamics (Carrillo et al. 2005). This parasitoid has some suitable characteristics for use in biological control, such as its short growth period (about 11 days at 25 ± 2 °C, 65 ± 5% RH, and 14:10 L:D), high fecundity of female wasps, long-term survival of adults in starvation condition, and adaptability to cold winter conditions (Alam et al. 2015).

Parasitoid wasps sometimes choose hosts for laying eggs that are unsuitable for the survival of their offspring (Heimpel et al. 2003). One of the reasons for this phenomenon in native parasitoid wasps is adaptation mechanisms to overcome host resistance (Yoon and Read 2016). In such a situation, the next generation of wasps has low survival and relatively weak establishment. These disproportionate hosts are called "evolutionary traps" (Schlenke et al. 2007). Evolutionary traps have a lot of ecological effects on the parasitoid population and eventually lead it to population extinction (Benoist et al. 2020). Therefore, from the point of view of biological control, changes in the selection and use of suitable hosts are very important for its establishment. The presence of an inappropriate host in the host complex of a parasitoid can affect the host–parasitoid population dynamics (Kaser et al. 2018).

Despite the importance of having information on the adaptation and establishment of *H. hebetor* for rearing in a laboratory, and in a release program for successful biological control, there is no information on the adaptation parameters, release conditions, and establishment of this parasitoid in date storage conditions for control of the date moth species including the Indian moth *P. interpunctella* and the dry fruit moth *E. kuehniella*. The aim of this study was to compare the ability of *H. hebetor* establishment in the population of two important date storage moths pests *P. interpunctella* and *E. kuehniella* and its role in their population fluctuations.

Methods

Hosts' rearing

Different developmental stages of the stored pests of date moths were collected by sampling infested dates from local date palm storehouses. Rearing of the two species *P. interpunctella* and the *E. kuehniella* was carried out at a temperature of 25 ± 2 °C and a relative humidity of 60 ± 5% in a growth chamber and in plastic containers covered by lids that had holes for ventilation covered with cloth fabric 40 mesh. Sayer dates were used for insectary rearing of the two species. A mixture of water and honey was used to feed adult insects during mating. In this method, the prepared cotton wick was placed in the tip of the micropipette and some of the water and honey prepared with a syringe entered the micropipette and it was attached to the wall of the breeding container (Bahmani et al. 2020).

Parasitoid's rearing

Initial population of the parasitoid wasps was obtained from an insectarium, affiliated with the local plant protection organization and reared on both host species. After being transferred to the rearing room, they were reared separately for five generations on larvae of the dried fruits moth and the Indian moth at the mean ± SEM temperature of 25 ± 2 °C, RH of 60 ± 5% and a photoperiod of 12:12, D:L. In this method, the homological populations were used from them for conducting experiments. The plastic containers, with dimensions of 8.5 × 7.5 × 9.2 cm, were used for rearing parasitoid wasps. For this purpose, the initial populations of parasitoid wasps were placed in these containers and their hatches were blocked using a 40-mesh white net cloth. Then, the host larvae of each species at the fourth and fifth instars were provided to the wasps on a sheet of paper in the container. Every 24 h, exposed larvae were replaced with new ones. The parasitized larvae were kept in the rearing room in containers until emergence of the parasitoid progeny. After five breeding generations, the required number of newly emerged wasps were collected from the respective colony with an aspirator and used in the experiments (Seraj et al. 2016).

Simulation of date storehouse conditions

Three glass cages (with dimensions of 40 × 50 × 50 cm) were made for each moth species. Nine holes (with a diameter of 5 cm) were installed on the body of each cage at equal intervals. These holes were completely covered with unlit cloth. Twelve kg of fruit dates of Sayer cultivar were placed in each glass cages. These dates were completely disinfested for 24 h at − 18 °C before transfer. Then, five hundred third instar larva of each species were transferred into each glass cage. A day later, fifty pairs of the newly emerged parasitoid wasps were released in each cage. The first sampling was collected 1 week after releasing the parasitoids and sampling continued for 24 weeks (Latifian et al. 2021).

Sampling method

Samplings were randomly done from each row of holes in the cage body. A sample consisted of one fruit with

an approximate weight of 15 ± 5 g. To prepare the samples, a solution containing 10 g of NaCl, 30 ml of distilled water, 30 ml of soda and a few drops of methylene blue was spilled. Each fruit was poured into an Erlenmeyer flask containing the solution. After boiling for 10 min, the contents of the Erlenmeyer flask were passed through a filter with a fine mesh of 0.5 mm in diameter. The filtered suspension was transferred to the decanter along with 100 ml of odorless oil. After 10 min, 1 cm of the intermediate phase + intermediate phase + 1 cm of the upper phase was transmitted to the Petri dish. Then, the number of healthy and parasitic larvae was counted using by stereomicroscope; since the wasp was an external parasitoid, it was possible to separate the parasitic larva by observing its presence on the host's larva (Latifian and Rad 2022).

Data analysis

The first step: calculating the percentage of parasitism by recruitment method (%P)

The method proposed by Van Drieshe was used for data analysis (Van Drieshe 1983). In this method, the initial average effective parasitoid population density (PSC), host (HSC) and percentage of parasitism (%*P*) are calculated by using Eqs. 1, 2 and 3, respectively. In these equations, P1, P2, L1 and L2 were the population density of parasitic larvae and the population density of non-parasitic larvae in two consecutive weeks. The number seven in Eqs. 1 and 2 is because the time interval between two samplings was 1 week (Sayad Mansour et al. 2011).

$$\mathrm{PSC} = \frac{\mathrm{P1} + \mathrm{P2}}{2} \times 7 \tag{1}$$

$$\mathrm{HSC} = \frac{\mathrm{L1} + \mathrm{L2}}{2} \times 7 \tag{2}$$

$$\%P = \frac{\mathrm{PSC}}{\mathrm{HSC}} \tag{3}$$

The second step: correlation between host and parasitoid population estimation

The correlation study is one of the methods used to monitor the population of pests and natural enemies during the season by examining the increasing or decreasing of the host and parasitoid population's densities. Time-series models were used to study the population dependence of two host moth species and the parasitoid wasp. They were assuming that the parasitoid population fluctuation was influenced by the host population fluctuation. The cross-correlation index (CCF) simulated the host and parasitoid population relationship. Host and parasitoid population variations may be independent or dependent at different times during the season. Even if the population fluctuation are different, with the modeled variance–covariance matrix and cross-correlation index, the growth rate of the parasitoid population over time could be evaluated based on the host species population. In this research, the time-series data of parasitoid and hosts population's densities were investigated based on the cross-correlation index (Pearman et al. 2008).

The third step: calculating the host and parasitoid population fluctuations rate

The host population (r_{H}) and parasitoid (r_{p}) fluctuation rates in the interval between two consecutive samplings are calculated by using Eqs. 4 and 5, and the average seasonal fluctuation rate of host and parasitoid population are calculated by using Eqs. 6 and 7. In these equations, $\mathrm{HSC}_{\mathrm{t1}}$, $\mathrm{HSC}_{\mathrm{t2}}$, $\mathrm{PSC}_{\mathrm{t1}}$ and $\mathrm{PSC}_{\mathrm{t2}}$ were the average of the effective population density of host and parasitoid in two consecutive samplings, respectively, and λ_{h}, λ_{p}, n are the average seasonal fluctuation rates of the host and parasitoid populations, and the number of samplings during the season, respectively (Entwistle and Dixon 1987).

$$r_{\mathrm{H}} = \frac{\mathrm{Ln}(\mathrm{HSC}_{\mathrm{t2}} + 0.01) - \mathrm{Ln}(\mathrm{HSC}_{\mathrm{t1}} + 0.01)}{7} \tag{4}$$

$$r_{\mathrm{p}} = \frac{\mathrm{Ln}(\mathrm{PSC}_{\mathrm{t2}} + 0.01) - \mathrm{Ln}(\mathrm{PSC}_{\mathrm{t1}} + 0.01)}{7} \tag{5}$$

$$\lambda_{\mathrm{h}} = \left(\sum_{i=1}^{n} r_{\mathrm{H}}\right)/n \tag{6}$$

$$\lambda_{\mathrm{p}} = \left(\sum_{i=1}^{n} r_{\mathrm{P}}\right)/n \tag{7}$$

Fourth step: calculating the host and parasitoid populations balance levels

The parasitoid (P^*) and host (N^*) population equilibrium points were estimated based on Nicholson's model by using Eqs. 8 and 9. In these relationships, (a) is the parameter of the parasitoid searching ability level, which is considered equal to 0.03 based on previous studies. Other parameters of similar equations were defined before (John et al. 2001).

$$P* = \frac{\log_e \lambda_{\mathrm{h}}}{a} \tag{8}$$

$$N* = \frac{\lambda_{\mathrm{h}} \log_e \lambda_{\mathrm{h}}}{(\lambda_{\mathrm{h}} - 1)a} \tag{9}$$

Fifth step: calculate the parasitoid establishment probability

The parasitoid establishment probability is consisting of the average probability of the parasitoid and host population density being above or below the equilibrium line during the season, respectively, which is calculated by using Eqs. 10 and 11, where parameters $P(x)_{\text{parasitoid}}$ and $P(x)_{\text{host}}$ were the seasonal probability of the parasitoid population being above the equilibrium line and the host population being below the equilibrium line, respectively. Other parameters similar to the equations have been defined previously (Stiling 1990; Mason et al. 2003).

$$P(X)_{\text{parasitoid}} = \sum_{i=1}^{n} (\text{PSC} - P*) \tag{10}$$

$$P(X)_{\text{host}} = \sum_{i=1}^{n} (\text{HSC} - N*) \tag{11}$$

Results

Seasonal fluctuation of the effective host and parasitoid populations and parasitism percentage

Seasonal fluctuations of the effective population of parasitoid and hosts are displayed in Fig. 1 through 24 weeks under laboratory conditions. The presence of host–parasitoid populations caused them to run into and created the parasitism phenomenon in the larval populations of hosts. Based on the conducted studies, two periods of activity were detected for the effective larval population of the two host species. The host populations started their activities shortly after release. The activity of *P. interpunctella* started about a week later than *E. kuehniella*. The population growth trends of two species had been increased during the first 6 weeks and in the 17th to 22nd weeks. The peaks of larval population density of two species were in the 10th and 21st weeks, respectively. At the beginning of the season and during the first and second activity periods, simultaneously with the increase of the effective population of the host (HSC), the effective population of the parasitoid (PSC) also increased with a delay of 1 week. Therefore, there was the extreme time opportunity in terms of phenology between the larval population of hosts and parasitoid for the occurrence of the maximum oviposition potential and parasitism action.

This difference in *E. kuehniella* population was more than that of *P. interpunctella*. As a result, the average maximum parasitism also decreased more in this population. The pattern of fluctuation in the parasitism percentage in the larval population of the two moth species was inconsistent with each other, and the parasitism percentage was higher in the larval population of *E. kuehniella*. In the larval population of moth, due to the slow start of the activity of the parasitoid in the second period of activity, the number of parasitized larvae decreased. Equally a result, the parasitoid population lagged in terms of phenology and therefore showed a lower parasitism percentage in the next stage compared to *P. interpunctella*.

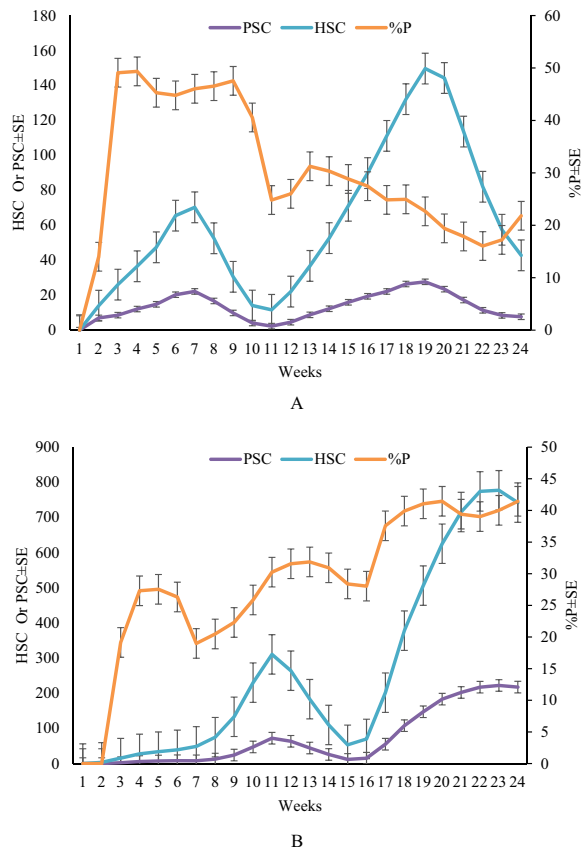

Fig. 1 Fluctuation curves of the effective parasitoid population (PSC) and the parasitism percentage (%*P*) and the effective larvae populations (HSC) of **a** *Ephesian kuehniella* **b** *Plodia interpunctella*

Investigating the correlation between parasitoid and host population fluctuations

The cross-correlation coefficients were compared in the study conditions according to Fig. 2 in order to investigate density-dependent functions between parasitoid and larval population of two moth's species. The association between larval *E. kuehniella* and *P. interpunctella* with parasitoid population was weak and negative at the first to sixth and the first to ninth weeks, respectively. In other words, the opposite relationship was detected between the fluctuations in the effective host moth larvae population density and parasitoid population density. A similar trend was observed between larval population

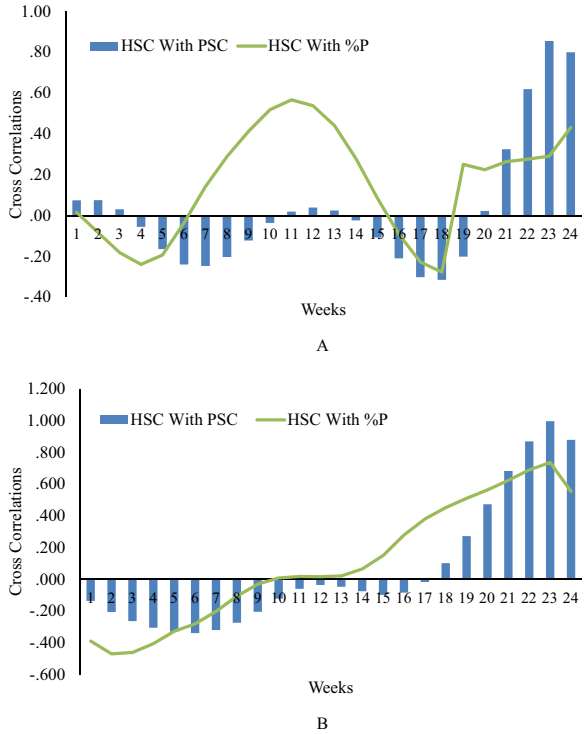

Fig. 2 Comparison of changes in cross-correlation coefficients between parasitoid (PSC) and host larvae (HSC) populations **a** *Ephestia kuehniella* **b** *Plodia interpunctella*) under release conditions

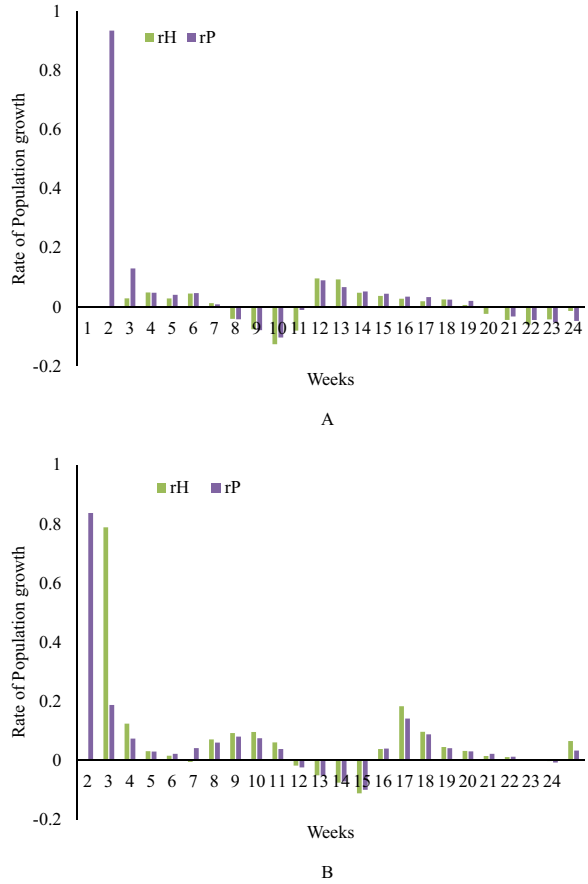

Fig. 3 Changes in the seasonal rate of the effective population of the parasitoid (r_P) (*Habrobracon hebetor*) and the host larvae (r_H) **a** *Ephestia kuehniella* **b** *Plodia interpunctella*

fluctuations and parasitism percentage. In the larval population of *E. kuehniella* and *P. interpunctella*, at the beginning of the seventh and ninth weeks, respectively, this correlation coefficient between their demographic parameters gradually increased and became optimistic. This positive trend continued for the *E. kuehniella* larval population until the 16th week. For the *P. interpunctella* population, it continued until the end of the experiment period, i.e., the 24th week. The maximum cross-correlation between the effective host population density *E. kuehniella* and *P. interpunctella* and effective parasitoid population density was observed in the 11 and 24 weeks, respectively. However in the conditions of *E. kuehniella* population, a new trend of fluctuation was recorded from the 19th to the 24th week, which continued with a moderate slope until the end of the season. Although, in this study, the number of larval generations of the hosts was not investigated, the number of population fluctuations was the same for both hosts.

Seasonal parasitoid and host populations' fluctuation rates

The seasonal parasitoid and host populations' fluctuation rates are shown in Fig. 3. The highest rate of parasitoid changes in the larval host conditions of both moth species occurred in the second week. Negative changes in the effective parasitoid population were also recorded in the eighth to ninth and twenty-first to twenty-fourth weeks for *E. kuehniella* and from the twelfth to the fifteenth week for *P. interpunctella*. The rate and direction of the fluctuation of the host population of two species larvae were in synchronization with the parasitoid. The lowest rates of host population changes in *E. kuehniella* and *P. interpunctella* larvae were in the 10th and 15th weeks and the highest rates were in the 12th and 3rd weeks, respectively.

Parasitoid and host population equilibrium level

The seasonal changes in the equilibrium level of the parasitoid and host larval population are presented in Fig. 4. The lowest level of parasitoid balance in the larval population of *E. kuehniella* and *P. interpunctella* was recorded in the 11th and 15th weeks, and the highest levels were recorded in the 20th and 24th weeks, respectively. During the 24 weeks of sampling, the equilibrium level of the larval population of the two host species was

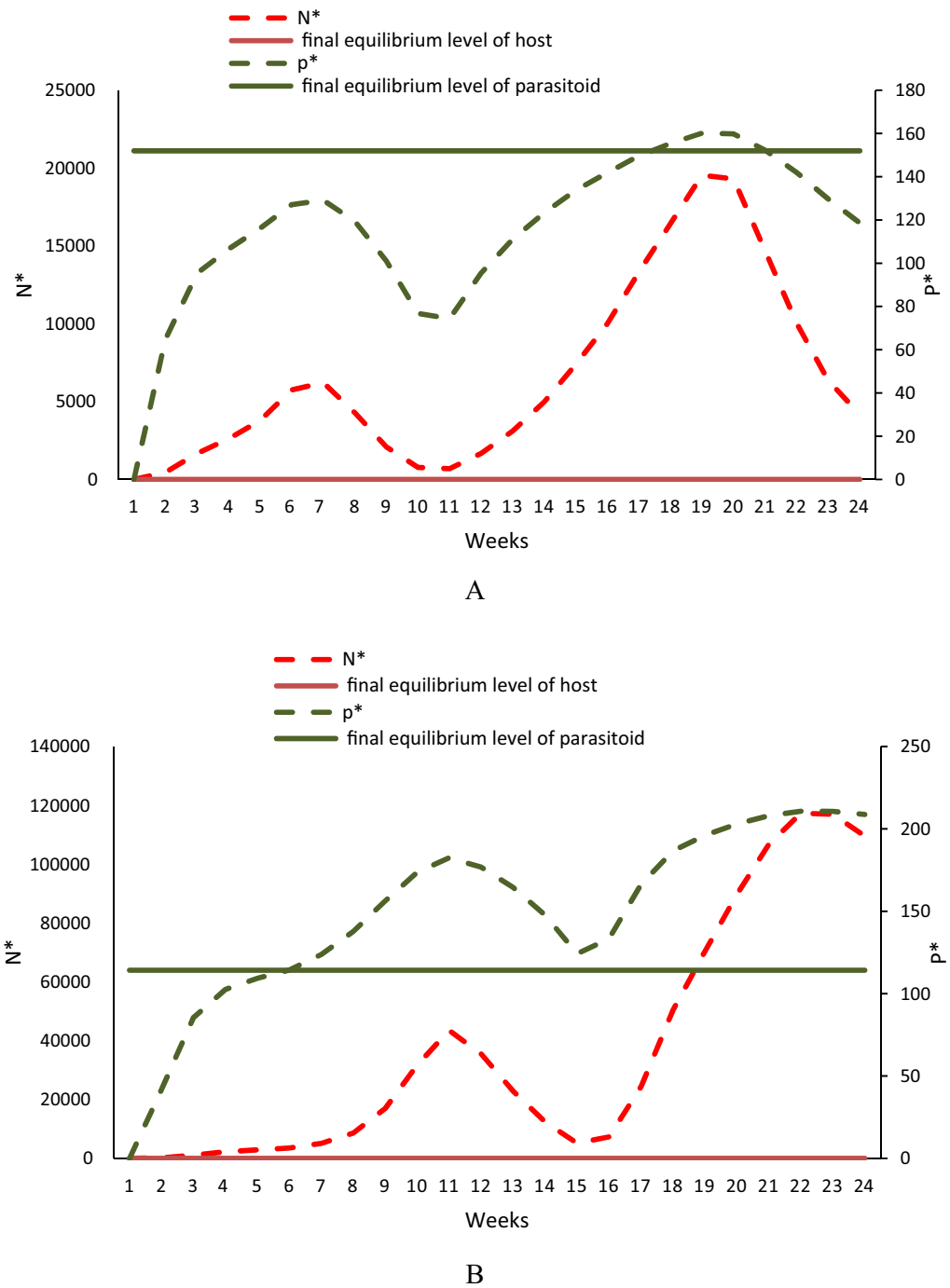

Fig. 4 Seasonal changes in the equilibrium level of the parasitoid population (*Habrobracon hebetor*) and host larvae population **a** *Ephesian kuehniella* **b** *Plodia interpunctella* (parasitoid (*P**) and hosts (*N**) populations equilibriums points)

always higher than the average seasonal equilibrium level. However, in the population of *E. kuehniella* and *P. interpunctella* larvae, the equilibrium level of the parasitoid population was higher than the average in the 17th to 20th and 25th to 24th weeks, respectively. The same trend and harmony were seen between the equilibrium level of the larval population and the equilibrium level of the parasitoid population. If equilibrium level of the host population was subordinate, then the difference between the equilibrium level of the host and the parasitoid and

the equilibrium level of the parasitoid population with its seasonal average was superior.

Probability of parasitoid establishment on host larval populations

The probability rate of non-establishment and deviation from parasitoid balance during 24 weeks of sampling is indicated in Fig. 5. In the conditions of release, the maximum probability rate of parasitoid non-establishment in *E. kuehniella* larval population was zero during the entire period and for *P. interpunctella* until the 22nd week. In other words, parasitoid establishment completely occurred in the larval population of two larval moth species. However in the *P. interpunctella* larval population, with the increase of the time interval of parasitoid release compared to the beginning of larval activity, the probability of the parasitoid population establishment going out of the equilibrium level increased. In these conditions, the probability of deviation from the balance of the parasitoid population was less than 12%.

Discussion

Identifying the conditions that increase parasitoid efficiency by increasing adaptation and reducing suboptimal host selection is significant in designing biological control. Avoidance of these suboptimal hosts (population extinction traps) increases their performance of biological control (Romero et al. 2020). However, there is little information on the effect of *H. hebetor* on date-stored moths, including *E. kuehniella* and *P. interpunctella*. A very important result of the present study is that the host species can affect the ultimate outcome of the efficiency and establishment of *H. hebetor*. This issue is very important in the mass rearing and release of this parasitoid for the control of *E. kuehniella* and *P. interpunctella* larvae.

The difference between the effective larval host populations and the lower effective parasitoid and the estimated percentage of parasitism showed higher value at the first activity period (1st to 14th week). In such conditions, the larval population of both hosts did not grow cross-sectional and on a limited time interval (moment constant) (Van Drieshe 1983), while the parasitoid had different developmental stages (larvae and pupae) on the host larva. Therefore, in the second generation, the effective host population showed more significant difference than the effective parasitoid population. The maximum percentage of parasitism in the population of *E. kuehniella* larvae was equal to 49% and it was verified in the 3rd week of sampling. From this point onwards, it had a downward trend, so that it reached about 16% in the twenty-second week. However, the percentage of parasitism in *P. interpunctella* larvae population after a decrease reached 19% in the seventh week, then with slight fluctuations until the end of the sampling period in the twenty-fourth week, it had an upward trend and reached 41% parasitism. However, the average percentage of parasitism observed in *E. kuehniella* (29.8%) was slightly higher than *P. interpunctella* (28.7%).

However, some significant differences were detected in the reaction depending on the abundance of *H. hebetor* in the population of larvae of two moth species *E. kuehniella* and *P. interpunctella*. The first point is that the speed of the parasitoid response to the host population increase showed a positive trend from the sixth week and thirteenth week in *E. kuehniella*, and *P. interpunctella,* respectively, so it was 7-week difference in time. However, the maximum cross-correlation coefficient, which indicates the strength of the density-dependent reaction, was equal to 0.74 and 0.57 in *P. interpunctella* and *E. kuehniella* larval population, respectively. The results of investigations by other researchers have shown that the conditions that were unrelated fluctuations in the host/parasitoid density were detected in the form of disturbances in the cross-correlation coefficient, which indicated a delay in the generation of the host compared to the parasitoid. On the other hand, if there is a strong mutation of positive correlation between the host and the parasitoid, which increased after a period of time and then returns to zero, it indicated the type III functional response of the parasitoid in the host population (Singh 2021). This state was verified in the conditions for the cross-correlation index between *H. hebetor* and *E. kuehniella* larvae between the 6th and 16th weeks. Contrary to these results, the risk of parasitism in *P. interpunctella* larvae was close to zero until the 10th week. From this point, it gradually reached its maximum value with a strong correlation trend, which indicated the type II functional response of the parasitoid in the host population (Singh 2021). Qualitative differences in the cross-correlation function provide a valuable tool for distinguishing between the functional responses mechanisms of parasitoids (Bešo et al. 2020).

Studies by other researchers have shown that parasitoids with density-dependent population growth have three different strategies in host selection: (1) parasitoids whose consumption rate is constant, (2) parasitoids change their activity to maximize access to unparasitized hosts in a generation, (3) parasitoids whose parasitism rates change between generations based on the relative density of hosts (Abrams and Tadeusz 1999). In this study, the attack rate of *H. hebetor* on *E. kuehniella* larvae varied from zero to 0.94 and on *P. interpunctella* larvae from zero to 0.84. Although the average rate of parasitoid changes in *P. interpunctella* was higher than *E. kuehniella*, the range of changes was lower.

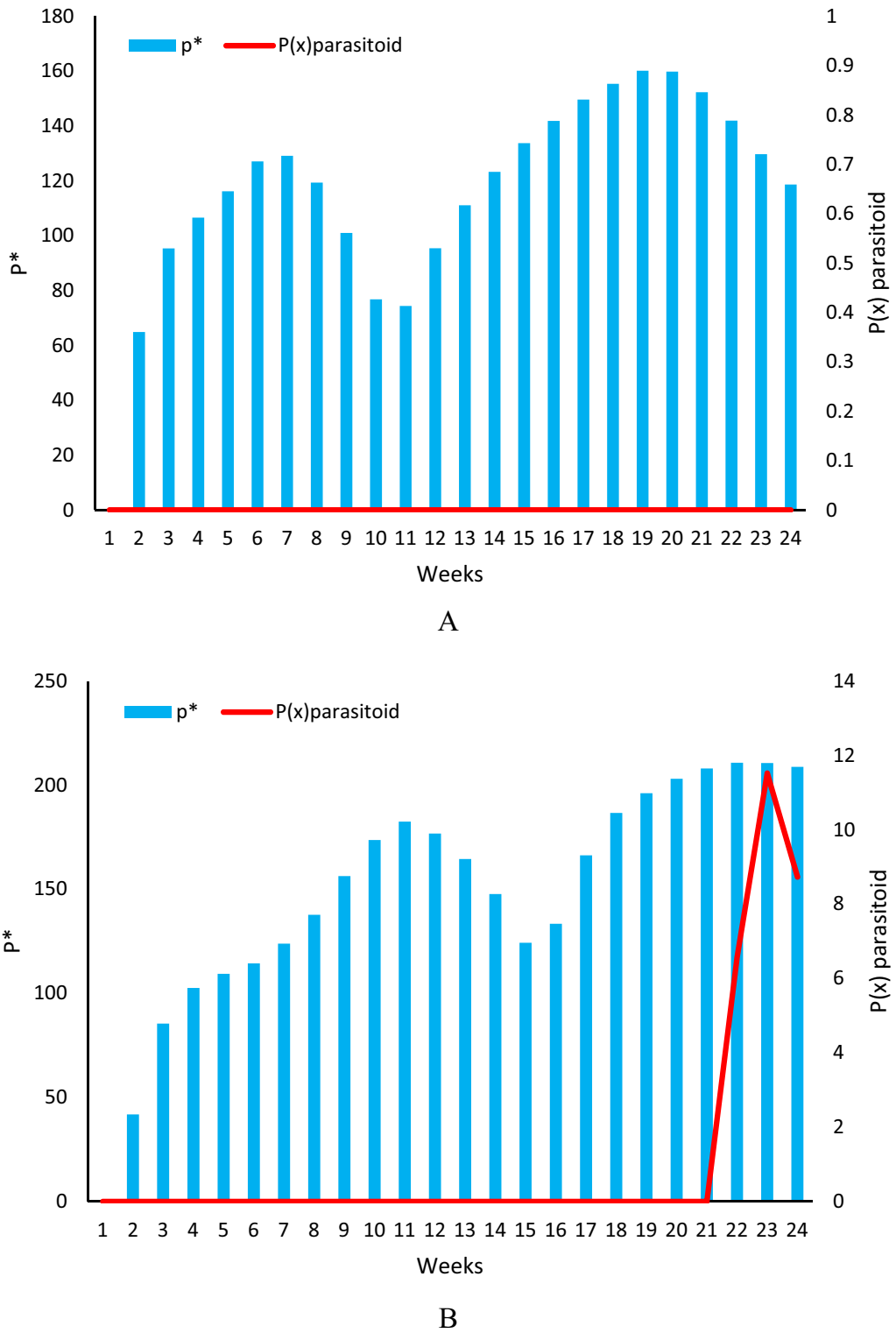

Fig. 5 Seasonal changes of the parasitoid population (*Habrobracon hebetor*) establishment probability on the larval host of **a** *Ephestia kuehniella* **b** *Plodia interpunctella interpunctella* (*P** and *P*(*x*) are equilibrium point and the seasonal probability of the parasitoid population)

Due to the high host–parasitoid correlation coefficient, both systems follow the pattern of parasitoids of the third group. Now the question is, do these adaptive processes in host–parasitoid population dynamics stabilize the system? Models show that this adaptive behavior often destabilizes population dynamics. The bigger average difference between the density of the non-parasitic and parasitic larvae inducted the longer instability. Therefore, in the *P. interpunctella* larval population, from the fifth week onwards, the level of the parasitoid population increased from its average seasonal equilibrium level, and this trend continued until the end of the sampling period. Nevertheless in the population of *E. kuehniella* larvae, where the difference between the average of non-parasitic and parasitic larvae was less significant, the population was a few overhead the seasonal equilibrium level only between the 18th and 20th weeks.

The optimal establishment strategy varies depending on the species and the different environments of the parasitoid (Madire and Netshiluvhi 2021). There is no information on the establishment conditions of *H. hebetor* release for control of *E. kuehniella* and *P. interpunctella* larvae. Studies by other researchers have publicized a negative relationship between the lack of host population and the success of parasitoid establishment. Because the probability of finding a suitable host and probability of establishment success was reduced coordinately (Cassey et al. 2004). This research showed that the probability of the non-establishment of the parasitoid *H. hebetor* in the population of *E. kuehniella* and *P. interpunctella* larvae was zero in the entire sampling period. The ratio of the unparasitized larvae to parasitized larvae population was even higher than that necessary for a complete establishment.

Conclusion

This study demonstrated that there was a strong correlation between establishment success and the severity of host and parasitoid populations in both studied systems. Prior to this research, no confirmed experiments related to the conditions of release and establishment of *H. hebetor* for the control of *E. kuehniella* and *P. interpunctella* in date storehouse conditions had been described. There is a need for information on the ecological relationship between this parasitoid and date storage pests to ensure the applicability of biological control and its proper efficiency. The information of this research is part of the findings needed in the development of biological control of *E. kuehniella* and *P. interpunctella* by *H. hebetor* in the storage conditions of date fruit. We examined hosts and parasitoid relationships to determine whether they influence the overall establishment rate of parasitoid released for biological control. Many traits appear to be important in influencing parasitoid establishment, but most are related to seasonal fluctuations in the host populations' densities. The date fruit of Sayer cultivar is the most popular industrial date in Iran. Therefore, the stored pest economic injury levels are higher, and it is possible to use biological control in storage warehouses condition.

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Availability of data and materials
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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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

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