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Optimization of a New Technique in Mass-Rearing of Insects Used in Biological Control of Important Pests

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ABSTRACT

In the mass rearing of parasitoid wasps used in the biological control of important crop pests, the method for producing hosts, especially moths, is essential. Nowadays, in the insectarium, collecting of moths reared in covers is carried by laborers and due to the scattering of moths' scales, respiratory problems are created for them. In this project, the traditional collection was replaced with a mechanized method, and a system consisting of two blower air transfer paths was designed and implemented on the insect preservation enclosure. So that with creating the air flow blown from the top of the cover and suction flow in the cover base, which is also used as air conveyor, insects are directed to the collecting container. Also, some arrangements were considered for uniformity of airflow distribution, flow rate control, and so on. The modeling was performed using the computational fluid dynamics method to study the fluid flow and estimate the air velocity. Since geometry changes in this type of simulation are simple, it is possible to examine various laboratory conditions before testing. In assessing the performance of the machine and investigating the amount of eggs laid by moths automatically collected, there was no significant difference between the new method and the traditional method. It indicated that the new method did not have a negative effect on the moth’s oviposition. Considering the implementation of the system, the development and optimization of this method are investigable for several interconnected covers. This technique can increase laborers’ health and facilitation so development of biological control and, consequently reduction of chemical pesticides.

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1. Introduction

Biological control refers to reducing the population of pests below the level of economic loss with the help of living and beneficial organisms in nature. Because it is entirely compatible with the ecosystem, it is one of the main components of integrated pest management (IPM). The history of the practical use of biological control in Iran dates back to about 50 years ago, which began with the mass rearing of *Rodolia cardinalis* (Mulsant) against the cottony cushion scale (*Icerya purchasi* Mask.) in the northern provinces, and the results were very successful. It caused the development of this technique by opening different insectariums in the country (Bellows and Fisher, 1999; Moghadasi, 2010). Gradually, the production of beneficial insects, especially *Trichogramma* spp. and *Bracon* spp., in the insect breeding centers (insectarium) under the supervision of the Plant Protection Organization was expanded. Although, until today, this production is done in the same way as in the past, while in the leading countries in biological control, new techniques have been used in mass rearing to increase production efficiency and reduce time and costs. Also, one of the most critical issues in the mass rearing of natural

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enemies is the mass breeding of intermediate hosts, which are generally species of moths. The way of rearing the intermediate host and using timing appropriate to the onset of the pest are essential points in the production of biological control agents such as *Trichogramma* and *Bracon* wasps. Also, the type of intermediate host that can be cultivated easily is essential.

In several countries, artificial eggs are used as intermediate hosts (Nordlund *et al*., 1997; Hajek, 2004, Cherif *et al*., 2021), but in Iran, usually, hosts such as Mediterranean flour moth, *Ephestia kuehniella* Zeller, or angoumois grain moth, *Sitotroga cerealella* (Olivier) is used (Moghadasi, 2010). In Iran, since 1984, the program of investigation and mass rearing of *Trichogramma* wasps and its intermediate hosts against pests of corn, cotton, rice, pomegranate and apple was carried out, and after that, various insectariums were constructed in different regions (Shojai *et al*., 1988; 1998). Since then, the mass-rearing of the intermediate host and the stages of moths and egg collection have been done manually and traditionally by workers, and there has been no change in the mass-rearing method. During this time, most of the studies are focused on the "identification" of different species, especially the *Trichogramma* wasps (For example, see: Ebrahimi *et al*., 1998; Poorjavad *et al*., 2012). In this way, the moths inside the rearing covers are guided daily to the bottom of the cover by a wind hose and collected in a container. This method causes many breathing problems for the workers, especially in the long term, due to the severe scattering of the moth scales. Considering the valuable technical knowledge of this issue and the reluctance to reveal new techniques in this field, no solution was found studying of the scientific sources to solve this problem. Targeted and mechanized use of airflow can be a possible solution to this problem. In the past, to collect insects or to sample their population in the field, suction traps or rear motorized propellers have been used (Taylor and Coleman, 1955; Dietrick, 1961; Taylor, 1962; Spiro and Weiss, 2003; Postali Parra and Coelho, 2022). Even the sensory receptors of wind flow in insects, which can detect airflow at low speed, have inspired the design of sensors or microelectromechanical systems (Ozaki *et al*., 2000; Krijnen *et al*., 2006). This study aimed to replace the traditional method with a new technique to optimize and mechanize the collection of intermediate hosts of biological control agents so that, ultimately it causes the ease of mass rearing, reducing costs and time of production without having an adverse effect on the oviposition rate so that the first steps of mechanizing this process in the country can be taken.

2. Material and methods

2.1. Insect samples

In this study, the angoumois grain moth was used as an intermediate host. For this purpose, barley seeds (*Hordeum vulgare* L. var. *Sahra*) infected with pest eggs were obtained from Dasht Naz agricultural company located in Sari. Infected barley seeds were poured into mesh frames (frames) with dimensions of 4 × 68 × 95 cm and the frames were placed inside the aluminum insect breeding cover. 10 kg of these seeds was poured into each frame, and nine aluminum frames were placed inside each cover.

2.2. Insect mass-rearing cover

The aluminum covers were made in a conical shape at the bottom to guide and collect the insects. The overall view and dimensions of the cover (in millimeters) are shown in Fig. 1. The frames containing infected seeds were connected to the top of the cover through a hook. Considering that the wall of the frames was a metal net, the moths were released from the frame after hatching and in a closed space inside the cover.

2.3. Automatic system design for a cover

After the initial studies, the use of two airflows was considered for the transfer of moths. In the automatic system, to separate and collect moths, positive and negative air pressure (thrust and suction) were used simultaneously. In front of the cover, a door made of a plexiglass Sheet was placed. A metal box was placed in the upper part of the cover and an electro-fan with forwarding blades was installed on top of it to create a blowing wind flow. The wind flow guides the moths inside the cover that come out of the frames and collect them. The connection between the fan and the mentioned box was through an accordion tube, and the opening was created on top of the pressure box. A perforated metal plate was used between the cover and the box above it to distribute the air flow relatively evenly inside the cover and between the frames. The diameter of each hole and the distance between the holes were 1 and 2 cm, respectively. The maps were prepared with CATIA software.

At the bottom of the frames, there were sheets with incomplete pyramid shapes like an inverted funnel. The end of the pyramid (smaller base) was connected to a diagonal tube through a rubber connection. On one side, this tube was joined by an accordion tube to an electrofan with backward blades - to create suction at the exit and as a winding conveyor to transfer moths - and on the other side was connected to the plexiglass cylinder tank to collect moths. Due to the transparency of the collection tank, its filling level could be seen during the work. To exit the airflow, the end of this tank was placed with a metal mesh. The advantage of using an air conveyor is the possibility of connecting several covers in series and transferring and collecting their moths at once. This issue is vital in insectariums that collect moths in several covers simultaneously. In this way, it was also possible to use the wind flow of the electro-fan above the cover for several adjacent covers by connecting them to the mentioned fan.

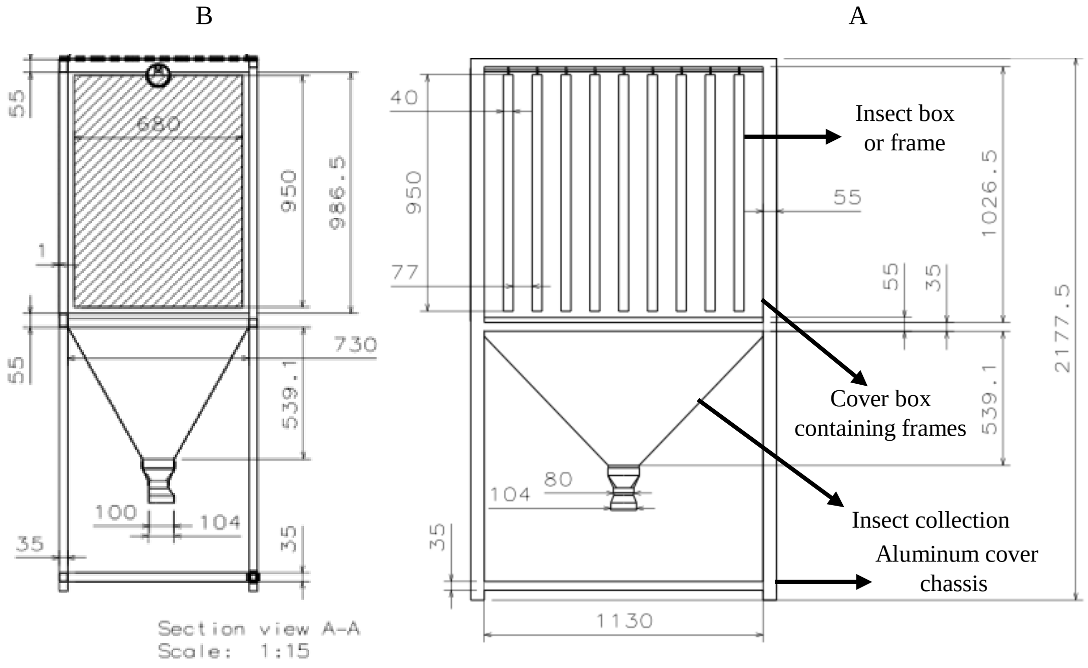

Figure 1. Dimensions of aluminum cover used for mass rearing of *S. cerealella.* Front view (A) and Cutouts view (B).

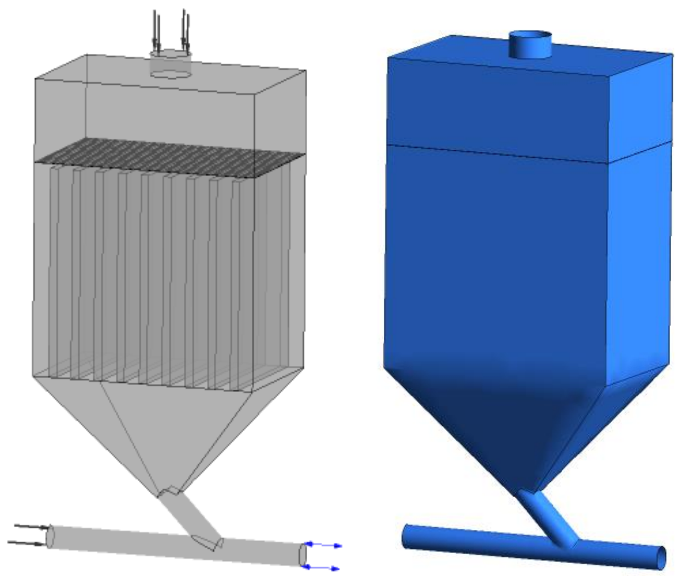

Figure. 2. Prepare geometry for simulation and loads of input and output in an aluminum cover.

To prevent moths from entering the fans, a layer of fabric mesh was placed under the perforated plate above the frames and also in the direction of the lower fan (at two points of the pipe). A control board was installed on the wall of the cover to control the speed of the fan motor and, as a result, the required speed and flow rate of the air flow. In the control panel, two inverters (each for one electro-fan) were placed. The input of the control board was single-phase and the output was three-phase, and changing the speed of the fans without significantly reducing the engine torque was done by changing the power frequency. City electricity, which has alternating current (AC), was converted to direct current electricity by a rectifier in the control panel, and then converted to three-phase alternating electricity, which is used in the electro-fan, through an inverter. In addition to the possibility of changing the speed of the engine propeller, the other advantages of using an inverter were the soft start of the engine and the reduction of energy consumption. It was also possible to change the torque in the used inverters. Through the plexiglass cover wall, the effect of airflow on the separation of moths inside the covers could be seen. For more strength of this wall, a galvanized gutter was connected from the outside to the front door of the cover. In order to collect insects, according to the population of moths in the cover at the desired times, it was possible to start the system once or twice (or more) a day. Also, it was possible to use the mechanized system continuously at low fan speeds (Fig. 1 and 2).

2.4. System evaluation

The oviposition rate of the female moths that were collected by the mechanized system was used to evaluate the system with the moths that were collected usually (traditionally) from inside the box. For this purpose, barley infected with moth eggs was poured inside the frames and aluminum cover under the conditions mentioned earlier. The temperature inside the aluminum cover was monitored with a temperature recording device (Data logger, TES 1384, data logger 4 Input Thermometer, Taipei, Taiwan) and varied between 20 and 28 °C from day to night. Also, the infected seeds were humidified daily with water spray so that the ambient humidity was maintained at 70-80%. After creating the optimal temperature and humidity conditions, about a week later, the moths emerged and it was possible to evaluate the system in actual situations. After collecting the moths from inside the mechanized system, 15 grams of moths were poured into ten funnels (with an opening diameter of 24 cm) and from the second day to the fifth day (4 days), the amount of laid eggs was checked and weighed. The mechanized collection was repeated three times separately and once again, done in a traditional and manual way. The oviposition funnels were kept in laboratory conditions (28±2°C, 40±10% RH and complete darkness).

2.5. Fluid motion simulation

Computational fluid dynamics was used to investigate the state of air flow movement inside the device and estimate it at different points. In this method, the equations of the problem, Navier-Stokes equations considering turbulent flows, are solved after discretization and by the finite volume method. The main equations used in this section are (ANSYS 2017):
Conservation of mass equation:

$$\frac{\partial \rho}{\partial t} + \frac{\partial \rho u_i}{\partial x_i} = 0 \quad (1)$$

Momentum conservation equation:

$$\frac{\partial \rho u_j}{\partial t} + \frac{\partial \rho u_i u_j}{\partial x_i} = -\frac{\partial p}{\partial x_j} + \frac{\partial \tau_{ij}}{\partial x_i} + f_i \quad (2)$$

In these equations, ρ, ui and p are fluid density, velocity and pressure, respectively, τ_{ij} is viscous stress, and f_i is gravity forces.

Turbulent flow is the movement of viscous fluid whose particles move randomly inside the flow field. In this type of flow, due to the intense mixing, the adjacent layers are at the same speed and all the characteristics of the fluid are continuously changing. Considering the importance of turbulence and its critical role, different and unique models have been presented to predict the nature and behavior of these types of flows. Some of these models have limitations for use, while some have vast applications and provide reliable results. Most models related to turbulent flow are based on the Reynolds-Averaged Navier-Stokes (RANS) Equations. In this research, the k-ε disturbance model was used. This model is generally used to simulate of airflow at normal speeds and provides suitable answers.

Ansys Workbench software system and specifically Ansys CFX software were used to construct the geometry of the device, meshing, problem definition, solution of the mentioned equations and examination of the obtained results. A number of 9118035 tetrahedral elements were made and the corresponding equations were solved in this domain using the finite volume method. Fig. 2 shows a view of the geometry prepared for simulation.

The wind speed created by electro-fans was measured and recorded in actual conditions to create the model. This work was done by changing the speed of the inverter in order to measure at different speeds. Airspeed was measured using a vane anemometer (AM-4201, Lutron Co., Taiwan). In order to solve the complexity of the model at the time of creating the system geometry, issues such as embedded nets to prevent the escape of insects, some air leakage from the seams, etc., were not considered. After creating the geometry of the system in the software, the speeds recorded at the output of the electro-fans were applied as the input speed of the model and the speed was estimated at different points of the system.

2.6. Statistical analysis

This experiment was carried out as a factorial experiment in a completely randomized design with ten replications. The method of collection (manual or mechanized) was considered as the main factor and the collection times (4 times: 2nd to 5th day) were considered as the second factor, and the amount of eggs laid in each funnel was considered as a repetition. Three times of mechanized collection were compared both individually and in general with manual collection. Comparing of the average eggs laid collected from the mechanized system with the amount of eggs laid by moths collected in the traditional method was done by One-Way ANOVA with Tukey's test at the 5% level.

Table 1. Analysis of variance of the amount of eggs laid by *S. cerealella*, collected 3 times mechanically (in general) and manually for 4 consecutive days.

S.O.V (Sources of variasion)	df (Degree of Freedom)	MS (Mean Squares)	F	P-Value
Type of moth collection	1	0.066	2.99 ^{ns}	0.086
Time	3	5.20	237.45 **	0
Type×Time	3	0.033	1.51^{ns}	0.212
Error	152	0.022		

ns and **: Indicate non-significant and significant at the 1% probability level, respectively.

Table 2. Analysis of variance of the amount of eggs laid by *S. cerealella*, collected 3 times mechanically (separately) and manually for 4 consecutive days.

Experiment	S.O.V	df	MS	F	P-Value
First collecting	Type of moth collection	1	0.336	16.63**	0
	Time	3	3.35	161.47**	0
	Type×Time	3	0.025	1.21 ^{ns}	0.31
	Error	72	0.021		
Second collecting	Type of moth collection	1	0.038	1.51 ^{ns}	0.223
	Time	3	3.18	126.9**	0
	Type×Time	3	0.40	1.57 ^{ns}	0.202
	Error	72	0.25		
Third collecting	Type of moth collection	1	0.24	0.96 ^{ns}	0.33
	Time	3	3.89	154.9**	0
	Type×Time	3	0.27	1.06 ^{ns}	0.36
	Error	72	0.25		

ns and **: Indicate non-significant and significant at the 1% probability level, respectively.

3. Results and discussion

The oviposition rate of moths was measured three times during mechanized collection and was compared with traditional collection separately and in general. The results showed that, in general, there is no significant difference in the amount of eggs laid by moths in these two methods. In other words, mechanizing the collection of moth -as a new technique- did not have a negative effect on moths oviposition rate (Table 1).

The results of each mechanized collection "separately" are compared with the results of the traditional collection and are shown in Table 2 and Fig. 3 As it is apparent in the table, the second and third mechanized collections did not have a significant difference on different days with the manual collection, although a significant difference was observed between the first mechanized collection and manual collection, and in general, the average of collected eggs in the first mechanized time was less than the manual or two other mechanized collection times. Considering that the test conditions were similar in all these tests, and on the other hand, no significant difference was observed between the other two mechanized collections and the manual collection, so the reason for the low oviposition rate in the first collection does not seem to be related to the collection method and is probably related to the biological conditions of the moths.

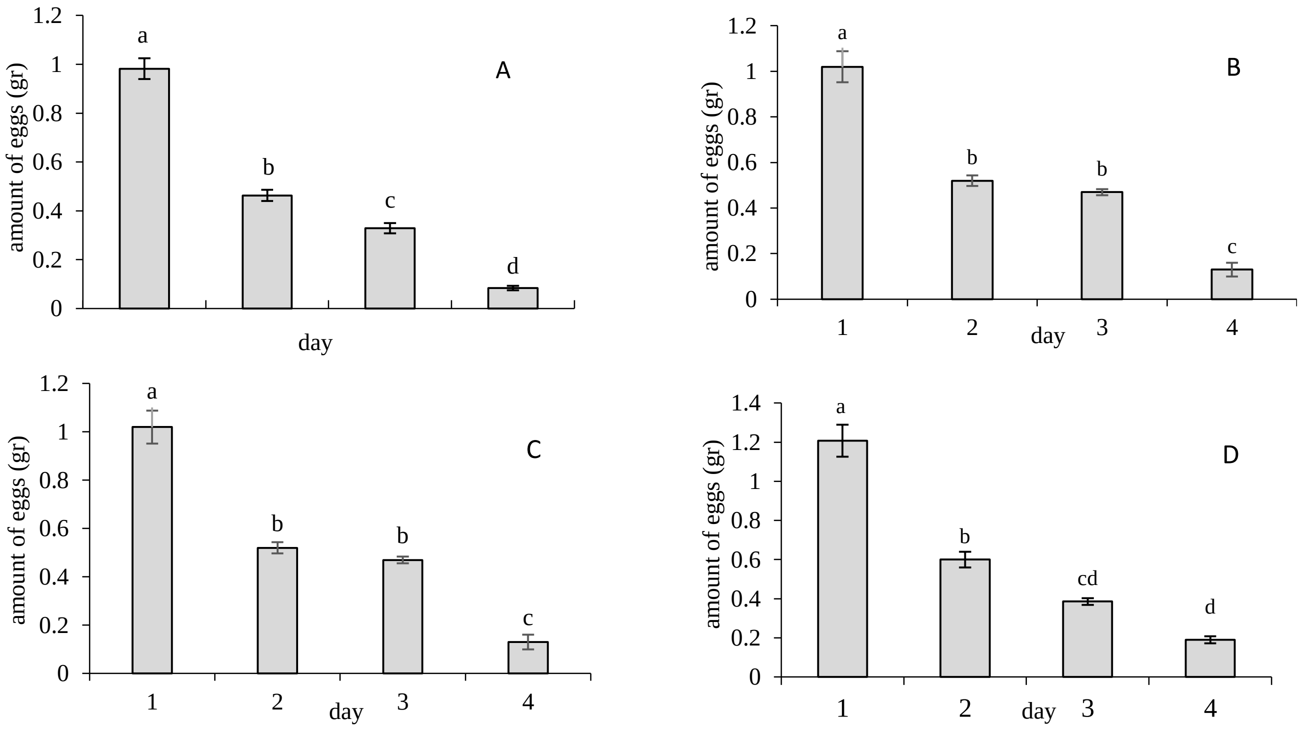

Figure.3. The amount of eggs laid by *S. cerealella* moths, in first (A), second (B) and third mechanized collecting as well manual collectin (D) for 4 consecutive days. Means followed by the same letter are not significantly different using Tukey's Test at P < 0.05.

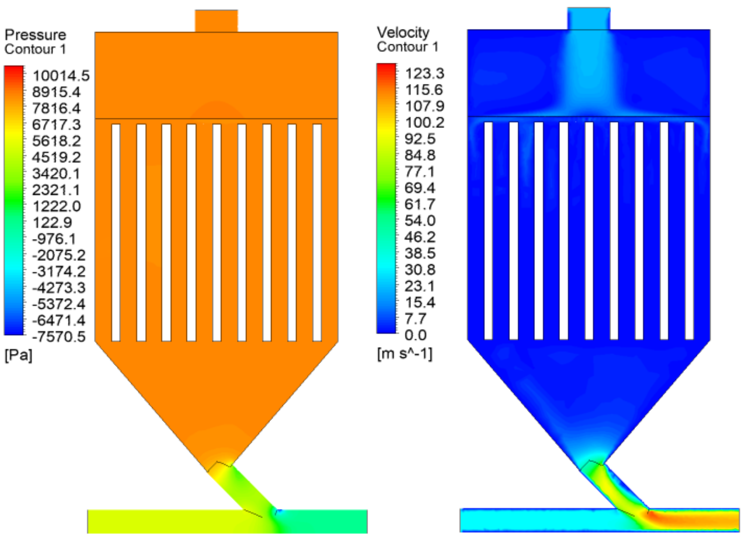

Figure.4. Distribution of air velocity and pressure in a propulsion system using CFD analysis.

A significant difference was observed between the collection times (second, third, fourth and fifth day) (Tables 1 and 2). The highest amount of eggs laid by the moths of each funnel was observed on the second day in the amount of approximately 1 gr. This amount decreased significantly every day in all three mechanized and manual collections (respectively, $F_{3,36}$: 203.51 , P<0.001, $F_{3,36}$: 81.7, P<0.001, $F_{3,36}$: 125.32, P<0.001 and $F_{3,36}$: 56.35, P<0.001) So that on the third and fourth day, almost half of this amount was recorded, and on the fourth day even much less (about 0.2 gr) was recorded (Fig. 3). The amount of eggs laid by moths in each funnel was about 2 gr on average for four days. According to the study of Akter et al. (2013) on the biology of the *S. cerealella* in the lab condition, each pair of male and female moths lays an average of 109 ± 57.23 eggs during four days. Considering that 15 grams of moths that were placed in each funnel contained 90-100 pairs of moths, approximately this amount of moths should be obtained from at least 11000 eggs, which is a little more than 2 grams obtained in the mentioned study. It seems that the reason for fewer amounts of eggs laid by moths in the present study is related to different environmental conditions, especially lower humidity in non-laboratory conditions; In the study of Akter et al. (2013), experiments were conducted in an environment with a relative humidity of about 70%, and this amount reaches up to 80% in insectariums, while in the current study, the relative humidity was 30-50%. Although these conditions were constant for mechanized and manual collection, and the results showed that there is no significant difference between these and the mechanization of the collection did not have a negative effect on the oviposition rate of moths, and therefore it can be replaced with the traditional method.

Wind flow, along with other factors that cause environmental stress, can affect different behaviors of arthropods and insects, such as migration, pollination, foraging, etc. (Weyman, 1993). Smaller insects need more adaptations to wind speed. For example, wind tunnel studies have shown that winds with a speed of less than 0.2 m/s affect the sitting and flying of *Prostephanus truncates* (Hom) (Fadamiro, 1996). Male wasps of *Aphidius nigripes* Ashmead, which are parasitoids of aphids, are unable to find their mates in wind currents with speeds higher than one m/s, and therefore their mating and female oviposition rates will decrease (Marchand and McNeil, 2000). The current study showed that the wind flow under the mechanized system has no significant adverse effect on the oviposition rate of *S. cerealella* females. However, in supplementary studies, the impact of different wind intensities on other biological and physiological characteristics of this moth can be investigated.

The results of CFD simulation are presented in Fig. 4. Due to the simplicity of this system in terms of fluids and the absence of components, reactions, combustion, and so on in this system, the data obtained from this simulation can be trusted with high confidence. Of course, this issue is possible by considering suitable conditions, such as no leakage, which should be considered in the supplementary designs. In this way, we can obtain the data related to the speed inside the main chamber, which we can't measure in the laboratory system. These results show the speed of the air flow inside the device point by point and its effect on the insects can be quickly investigated. Although there has been no study on the impact of wind speed on this species, according to the results of oviposition rate and the values of wind speed inside the system, it seems that the effect of wind on this insect in the built system is not significant. Fig. 3 shows the speed distribution and pressure distribution in the propeller system. Fig. 3 shows the distribution of speed and pressure in the moth collection system. Despite applying a speed of up to 21 m/s in the upper entrance of the system, the maximum speed inside the main chamber is less than five m/s and this wind speed is tolerable for most insects (Spiro and Weiss, 2003). Also, there is a balanced distribution of speed and pressure inside the system and local or sudden increase of speed or pressure is not seen inside the device.

4. Conclusion and suggestions for the development and improvement of the system

Considering that many aluminum covers are usually used in insectariums, at the beginning of this research, the possibility of developing the system for more covers was considered. In the current designed system, it is possible to increase the length of the airflow ducts at the top and bottom of the covers and connect several covers by feeding from the same fans.

In general, in this study, blowing and suction forces were used to collect rearing moths in the biological control of pests. It was tried to significantly reduce the interference and direct contact of humans with the scales of moths. In the subsequent studies, it is suggested to add a steering wheel and investigate other methods for air distribution on top of the cover. With the help of computational fluid dynamics that was used in the simulation of the moth collection system in this research, the fluid velocity inside the device can be estimated. Since the geometry change is done quickly in this type of simulation, It is possible to check different laboratory conditions before conducting experiments. Therefore, it is suggested to use this method in making similar devices or investigating the effect of wind speed on insects.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not to use any patients in this research.

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