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Development of AI Based Larvae Transfer Machine for Royal Jelly Production

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ABSTRACT
Honeybees produce many different products beneficial to humans. One of these of is royal jelly which is the bee product with highest nutritional value but is most difficult to produce. The most time-consuming procedure in royal jelly production involves removing larvae with ideal size from the honeycomb cells and transferring them to queen cups. In order to increase the speed of the larva transfer process and perform it without labor power, a machine autonomically performing larva transfer was developed in three stages. Firstly, a CNC platform that can move on three axes above the honeycomb was created. In the second stage, a camera device was developed to image the larvae and mounted on the platform. Later larvae were photographed with this device and labelled. Tagged photos have been quadrupled by data augmentation methods. A Mobiledet+SSDLite deep learning model was trained with these photographs and this model identified larvae with ideal size with 96% success. Additionally, the central points of the honeycomb cells were identified with the Hough circles method. In the third and final stage, a device which can transfer the identified larvae from the honeycomb cells to the queen cups was developed and mounted on the platform. Later general software controlling the platform and devices was developed. At the end of this study, for the first time in the literature, an artificial intelligence-supported machine was developed for automatic transfer of ideal larvae from natural honeycombs for royal jelly production.

Keywords: Bee larvae transfer, Image processing, Mobiledet SSD Lite, Royal jelly

1. Introduction

As a result of apiculture activities, led by honey, pollen, beeswax, propolis, honeybee venom and royal jelly are produced (Alvarez-Suarez 2017). Royal jelly is a produced by the hypopharynx of bees as a result of transfer via blood of the nutrition from honey and pollen consumed by 4-12-day-old young worker bees after digestion in the digestive organs (Pirk 2018; Pereiraet al. 2019). It is one of the most important products among bee products (Yeung & Argüelles 2019). At the same time, it is the bee product with most difficult production and highest price (Bruneau 2017).

Royal jelly is used in health products, for healthy nutrition and in the cosmetic industry (Ramadan & Al-Ghamdi 2012). Clinical tests and studies researching the effects of royal jelly on a variety of diseases obtained positive results for treatment of many diseases like cancer, diabetes, ulcers, infertility, hypertension, tumors, etc. (Bincolettoet al. 2005; Silici et al. 2009; Shirzad et al. 2013; Miyata & Sakai 2018; Ahmad et al. 2020; Sofiabadi & Samiee-Rad 2020). Royal jelly is used for different purposes in the food, health and cosmetic industries in many countries. Studies investigating the effects on health stated it has positive effects like antibacterial, antimicrobial, anti-inflammatory, antioxidant, antihypertensive, antiseptic and antitumor properties (Okamoto et al. 2003; Fratini et al. 2016; Park et al. 2019). Additionally, royal jelly was revealed to strengthen the immune system and have age-preventive effects in different studies (Vucevic et al. 2007; Kunugi & Mohammed Ali 2019).

Royal jelly is a yellowish-white and acidic secretion from the hypopharyngeal and mandibular glands of nurse bees used to feed young worker larvae during the first three days and the queen bee during her entire life (Ahmad et al. 2020). For royal jelly production, firstly artificial queen cells called queen cups are created. Later, larvae hatched 1-2 days before found in normal honeycomb cells are transferred to the queen cups (Gençer & Fıratlı 1999). The larvae are examined for size 1-2 days after hatching. The reason for use of larvae hatched 1-2 days before is that the production amounts for royal jelly are highest for larvae of this size (Gemeda et al. 2020).

For royal jelly production, production increases in proportion to the number of larvae that can be transferred and the number of hives used. However, the process to transfer the larvae by hand from the honeycomb to the queen cup lasts 5-15 seconds (Grafting 2016). The duration and quality of the procedure are affected by factors such as manual skills, location of the larvae in

the honeycomb and fatigue. In the apiary where the study was performed, nearly 2000 larvae can be transferred in one day. Considering that one person may transfer one larva in mean 10 seconds, this job requires 5-6 working hours without breaks.

In order to transfer larvae for royal jelly production, firstly it is necessary to identify the honeycomb cells and larvae with image processing methods. There are multiple studies about image processing in the agricultural field for classification and detection of marks on fruit like apple, pear, etc., determination of the placement of fruit on branches, and detection of the dimensions and quality of agricultural products (Dubey & Jalal 2016; Capizzi et al. 2016; Yang et al. 2017; Zhang et al. 2017; Ponce Aquino & Andújar 2019; Songaet al. 2020). In the apiculture field, there are image processing studies related to counting bees, determining the gender of bees and observing bee movements (Dembski & Szymański 2019; Ngoa et al. 2019). Additionally, there are studies related to classification of honeycomb cells, detection of size and detection of only large larvae present in the cells in the literature (Sparavigna 2016; Alves et al. 2020; Giraud 2020). However, there is no study related to identification of larvae with sizes for use in royal jelly production. This study has the feature of being the first in the literature from this aspect.

In this study, a machine is proposed to autonomously complete larva transfer to accelerate and increase royal jelly production and lower costs. This machine is a platform that can move on 3 axes with a camera mounted on the platform, a larva transfer tool and an integrated system providing control. The system detects larvae with ideal size with computer imaging and the larva transfer tool is controlled on the platform which can move over the honeycomb to remove larvae from the honeycomb and transfer them to queen cups.

2. Material and Methods

2.1. Larvae transfer system setup

The larva transfer system comprises 3 sections. These are a mechanism that can move in 3 axes, a camera setup linked to this mechanism which can image the larvae and a tool to transfer the larva. The schematic for the larva transfer system is presented in Figure 1.

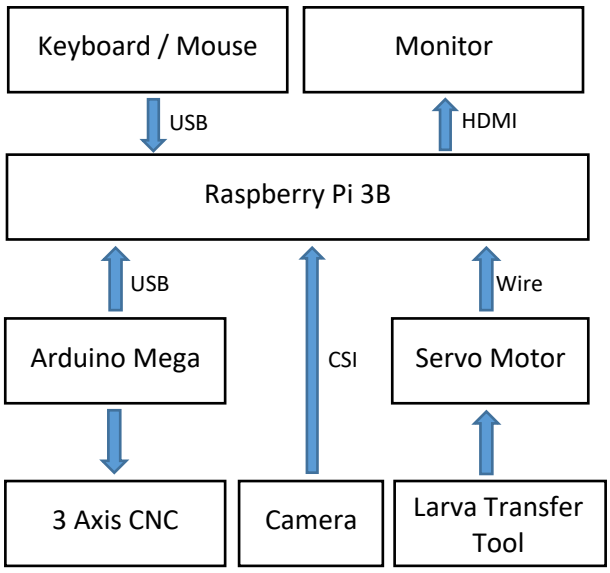


Figure 1- Schematic for larva transfer system

2.2. Triaxial mechanism

A system which can move in 3 axes above the honeycomb was developed to identify and transfer the larvae in the honeycomb. Design of this system noted the standard honeycomb frame size and dimensions of the rod where the queen cups are located. Images of the natural honeycomb dimensions and the placement of the mechanism above the honeycomb and cup rod are given in Figure 2.

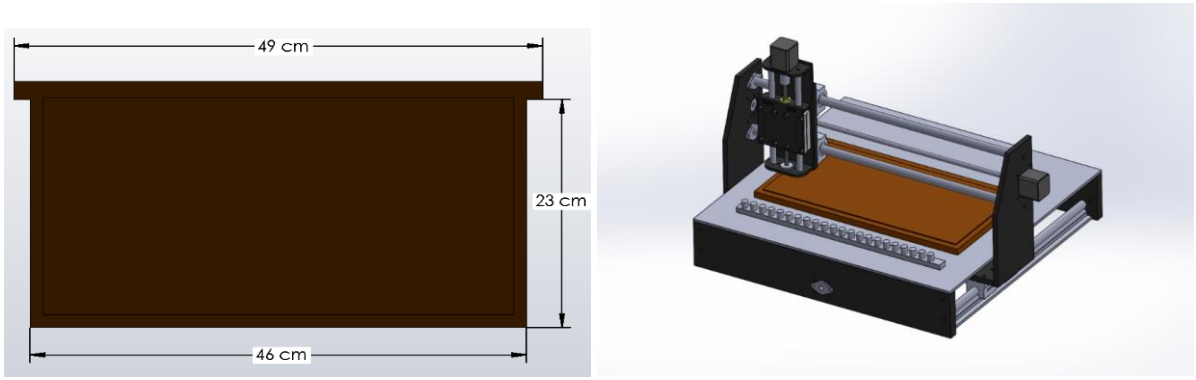


Figure 2- Honeycomb frame dimension and system design

The system used a classic cartesian movement mechanism. Movement on the X, Y and Z axes was provided by a belt and pulley mechanism powered by Nema 23 step motors for the X and Y axes and a Nema 17 series step motor for the Z axis. To drive the motors, TB6560 series step motor driver cards were used with control by an Arduino Mega microcontroller (Arduino Mega 2560 Rev3). The combined form of the system is given in Figure 3.

Figure 3- The combined form of the system

The Arduino Mega embedded system, which drives the motors for the movement of the platform, was run on the GRBL software. GRBL is an open-source coded, embedded, high performance g-code-parser and CNC milling controller written in optimized C that will run on a straight Arduino (grbl). When the system is to be moved, G codes are sent to the Arduino Mega through serial connections and the system moves to the required point.

2.3. Imaging area

A camera was mounted on the mobile head of the system which can move in 3 axes for use to image the larvae. Many alternatives were attempted for the camera (Go Pro Hero 5 Black, A4Tech Pk-910P 720P WEB CAM, Microscope Cam 500X); however, due to the small dimensions of the larvae (~2 mm) and the deep and dark honeycomb cells, cameras with automatic focus could not clearly photograph of larvae. For this, a 5 MP resolution camera operating via the camera serial interface with manually adjustable zoom features was used (Raspberry Pi Camera). The first photographs taken with this camera could not view the larvae in the dark-colored cells. The reason for this was the deep and dark honeycomb cells. For this reason, a lighting LED was added to the system. The mounted view of the camera system is presented in Figure 4.

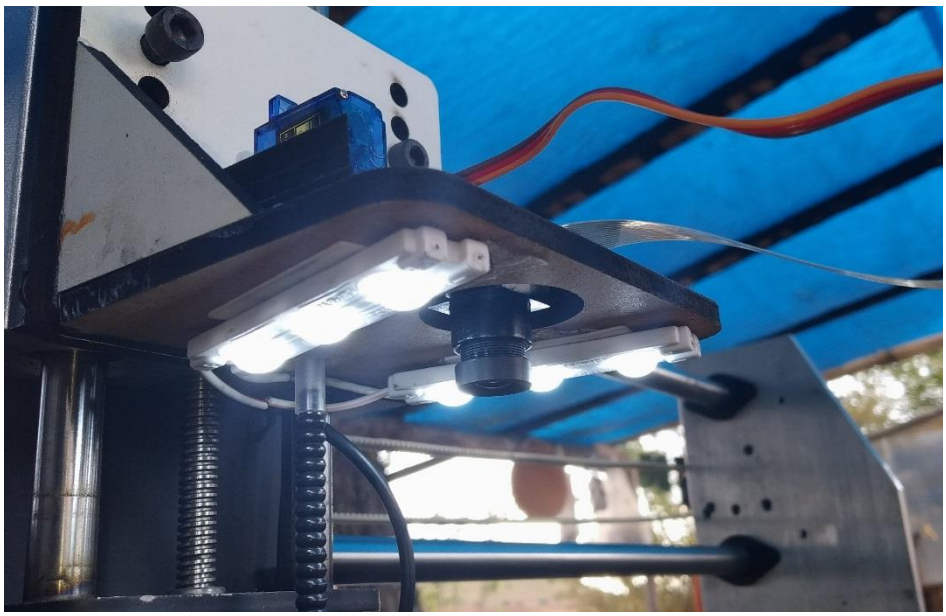


Figure 4- Camera and lighting LED

In this stage to operate the software providing imaging with the camera, platform movement and all controls, a Raspberry Pi 3B was added to the system (Raspberry Pi 3 Model B).

2.4. Larva transfer tool

Larvae are transferred by hand with a specialized tool similar to a ballpoint pen shown in Figure 5. This tool is first placed in the honeycomb cell and slowly moved to scoop the larva onto the tip of the tool. Due to the adhesion of the royal jelly surrounding the larva, they stick to the tip of the tool. Then, the larva is brought to the queen cup and the button on the other end of the tool is pressed to push the tongue forward and drop the larvae into the queen cup. Thus, the transfer process is completed (Grafting 2016).

Figure 5- Larva transfer tool

The larva transfer tool was mounted with the camera on the mobile platform in the study. Operation of the larva transfer tool was completed with an arm linked to the shaft of an RC servo motor. This mechanism can press the button on the tool and drop the larvae. The appearance of the larva transfer tool on the mechanism is shown in Figure 6.

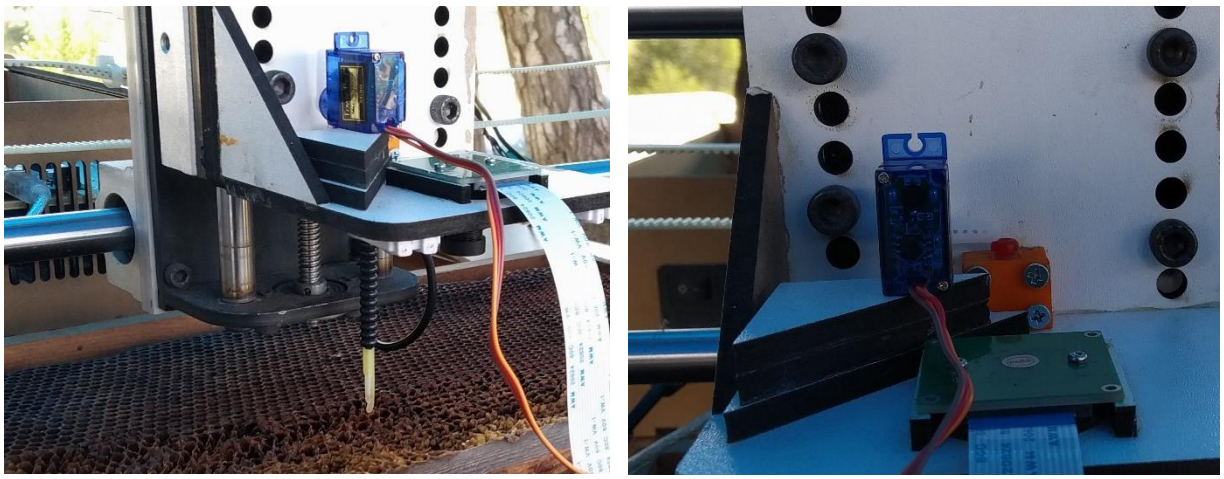


Figure 6- Appearance of larva transfer tool on the mechanism

3. Larva detection and transfer

In order to perform the transfer procedure, firstly it is necessary to identify the larva within the honeycomb cells. Then it is necessary to place the larva transfer device at the point where the larva is identified, take the larva from the cells and finally leave them in the queen cups.

3.1 Photographing and detecting larvae

For detection of larva, photographs were taken of both sides of 25 different honeycombs. A total of 1356 photographs were taken from different regions in each honeycomb. The camera was placed at 10 cm distance from the honeycomb in order for the system to clearly image 65 honeycomb cells. The best focusing and rate of honeycomb cells in focus was obtained from this distance in the photography process. For the photography process, honeycombs that were both old and new were chosen and that contained dense larvae. The new honeycombs were yellow in color, while they darkened over the years to reach a color close to black. Figure 7 shows photographs of old and new honeycombs.

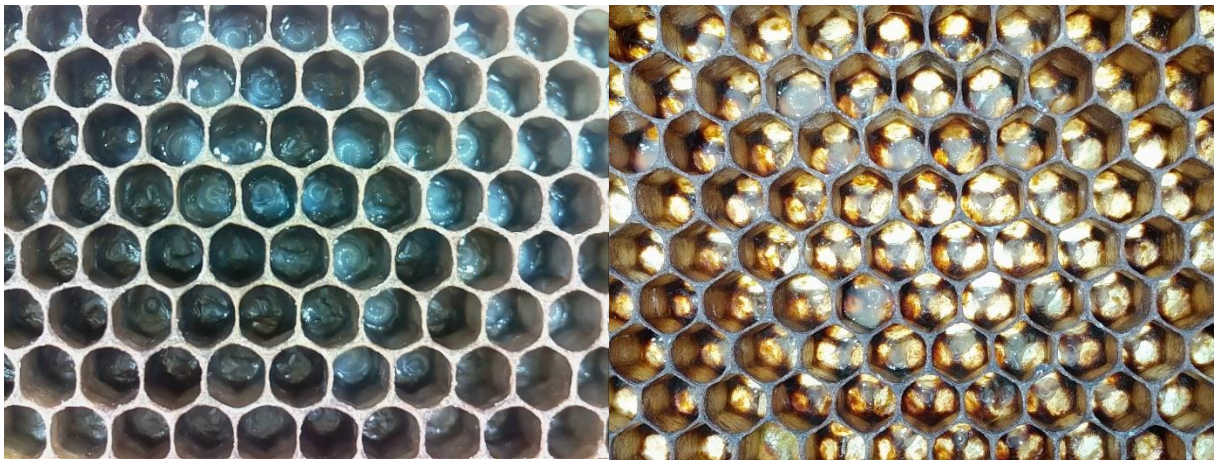


Figure 7- Larvae in honeycomb cells

When honeycombs are first created by bees, they have a yellow color and have pronounced rates of transparency. In this situation, it is difficult to distinguish the white-colored and small larvae from the background color. Additionally, as the honeycombs darken, the light rate in the cells containing larvae reduces and makes photography difficult. Honeycombs with different colors were photographed for the system to operate in all these different conditions.

The resolution of the photographs was 640*480 and 96 dpi. The resolution was low in order to use a computer with low system resources, like Raspberry Pi, for the larva detection process in the system.

The larva detection process is actually an object finding process. Currently one of the most commonly chosen object finding methods is deep learning-based object identification. The general feature of these methods is that they find the desired objects

themselves after being trained in the distinguishing features with many samples. Generally, these deep learning methods use convolutional artificial neural networks (O'Shea & Nash 2015; Albawi et al. 2017; Agarwal et al. 2019).

CNNs (Convolutional Neural Networks) are generally designed to consist of convolution, sampling and full link layers and work by performing operations in these layers respectively (Amidi & Amidi 2019). In the convolution layer, some filters are applied to the image to extract low- and high-level features from the image. In the pooling layer, methods such as max and avg are applied to the data, reducing the size of the data and reducing the number of transactions that need to be made on the network. In the full connection layer, there is a neural network in which every data coming out of the pooling layer is given as input to all neurons and is in principle the same as a traditional multilayer perceptual neural network (MLP). This neural network performs the image classification process.

There are many available deep learning-based object finding models and studies in this area continue intensely. However, as the imaging process in this study used Raspberry Pi 3B, it was necessary for the model to be used for the platform to be operable in systems with low processing power.

Currently, there are many object finding CNN models that can be used with Raspberry Pi. Examples of these include SSD (Single-shot Detector), Faster R-CNN and YOLO (You Look Only Once) (Zhang et al. 2018; Chandan et al. 2018; Huang et al. 2018; Foley & R, 2018). Studies comparing the operating performance of these models observed successful object detection and accurate location with many algorithms according to the dimensions of the photographs, dimensions of the objects and training durations (Desai et al. 2020; Bouguettaya et al. 2019). When performance with Raspberry Pi is considered, the MobileNet+SSDLite-based models were identified to produce better results (Gunnarsson 2019; Brokate 2019; Huang et al. 2017). Within the scope of the study, one of the most up-to-date and successful versions of these models of Mobiledet+SSDLite was used (Xiong et al 2021).

MobileDet is one of the most advanced image classifier model architectures for mobile devices that emerged with the work of University of Wisconsin-Madison and Google researchers (Xiong et al. 2021). MobileDet uses three main blocks in the design of its backbone: inverted bottleneck (IBN), fused convolution, and Tucker/CP decomposition. In this study, a pre-trained deep learning model SSDLite MobileDet-CPU (ssdlite_mobiledet_cpu_320x320_coco) was used. The structure of this model is presented in Figure 8.

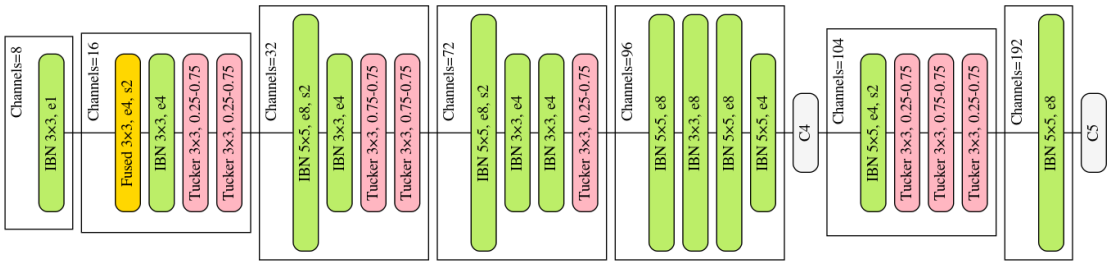


Figure 8- Mobiledet CPU Architecture

In order for Mobiledet+SSDLite to be able to detect larva, it is necessary to train the model with images of larva from the honeycomb photographs. For this, a total of 6373 larvae with ideal size for royal jelly production were labeled on 640*480 photographs taken of the honeycombs with the Labellmg software (LabelImg). The labelling process is presented in Figure 9.

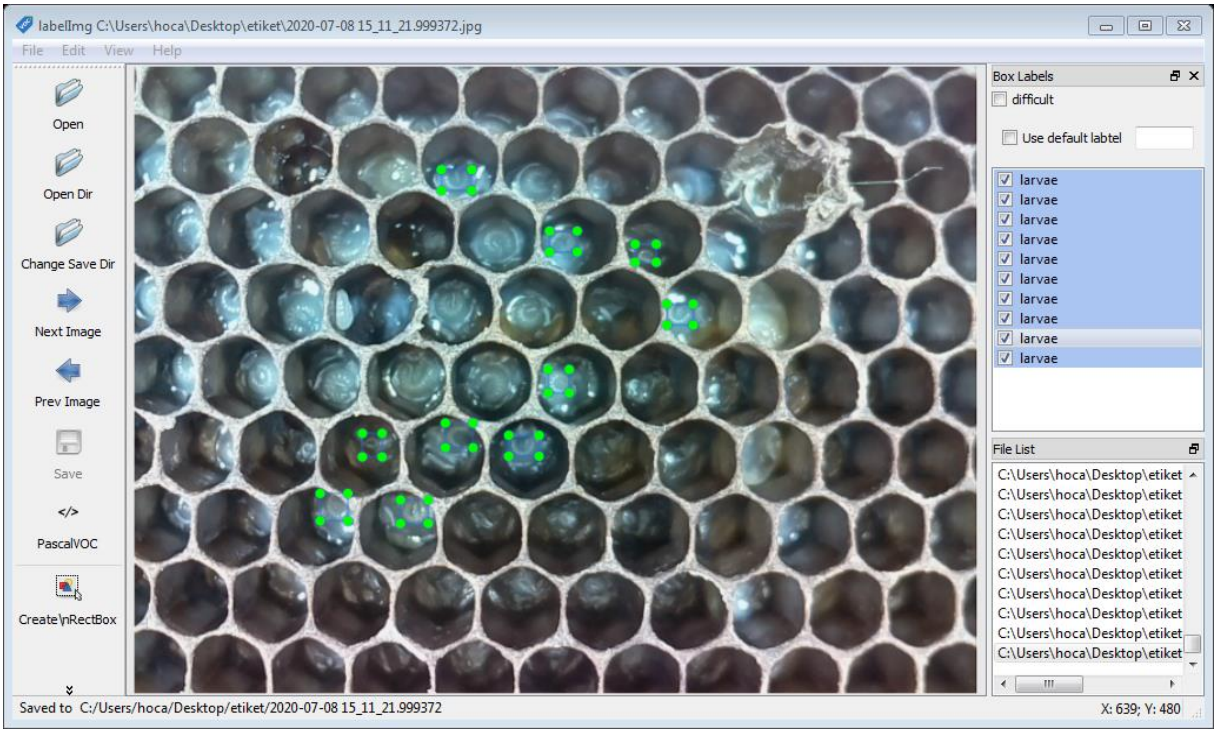


Figure 9- Labelling of larvae with ideal dimensions

In order to increase the success rate of the model to be trained after the labeling process, the data augmentation process was applied to the data set in our study. Using data augmentation techniques, a relatively smaller dataset is transformed into a larger database and deep learning algorithms are trained with these datasets. The basic principle in the data duplication is generate additional training data by applying some deformation, rotation, translation etc. to the existing data (Dieleman et al. 2015; Salamon & Bello 2016). There are many data augmentation techniques, such as rotating the image at different angles, *horizontally vertically*, adding noise and color manipulation to the image. In this study, in order to increase the data, all the marked photos were rotated 90, 180 and 270 degrees, also considering the shapes of the larvae on the honeycomb, and the tag files were automatically changed with the written python script. With this process, the number of photos increased to 5424 and the number of larvae to 25492.

70% of tagged photos are reserved for training, 20% for verification and 10% for testing. Augmented data was used to in the separation process. Images were randomly selected from each honeycomb in the proportions specified for each data set. The training process was carried out using TensorFlow framework and Google Colab platform. The graphics processor used is Tesla P100 GPU. The model is trained using stochastic gradient descent with an initial learning rate of 0.001, 0.9 momentum, 0.0005 weight decay, and batch size 32. The training was carried out in fifteen thousand steps. As a result of the training, the success rate on the validation data was calculated as 96%.

The trained model, after the training process was tested to measure the performance of the model with all the photos reserved for the test. In this test process, 2439 larvae in 542 photographs were tried to be detected and 2347 of them were successfully tested. In addition, 45 false larvae were detected. Accuracy, f1-score, precision, recall values are also used to measure the performance of CNN-based models (Sokolova & Lapalme 2009). To calculate these values, True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN) values were determined and the formulas in Table 1 were used.

Table 1- Model results

Metric	***Equation***	***Values***	***Results***
Accuracy	$\frac{TP+TN}{TP+FN+TN+FP}$	$\frac{2347+0}{2347+93+0+45}$	0.962
Precision	$\frac{TP}{TP+FP}$	$\frac{2347}{2347+45}$	0.981
Recall	$\frac{TP}{TP+FN}$	$\frac{2347}{2347+93}$	0.961
F1-score	$\frac{2*precision*recall}{precision+recall}$	$\frac{2*0.981*0.961}{0.981+0.961}$	0.971

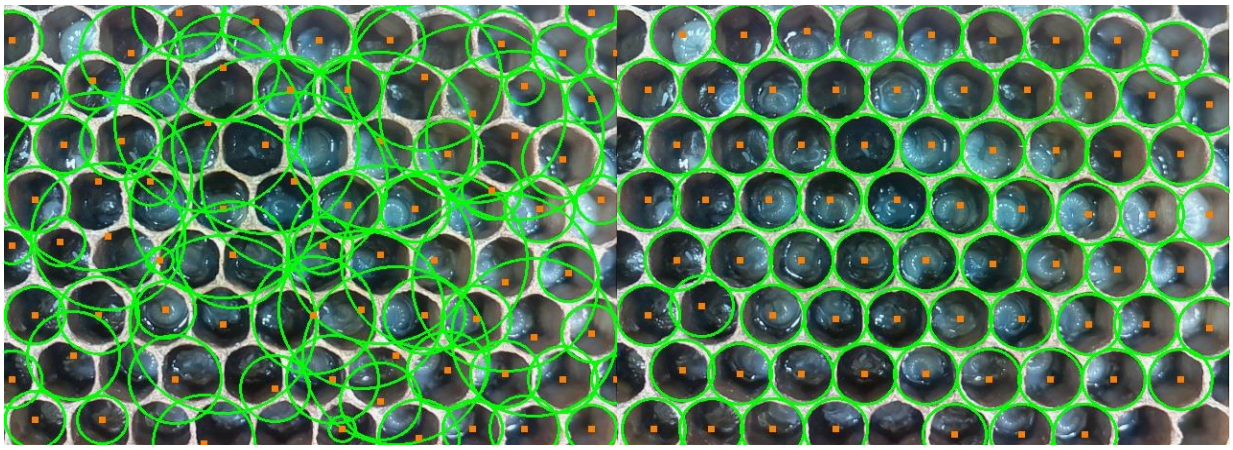
The Mobiledet+SSDLite model used in the study was more successful than the Faster R-CNN model developed by Gungormus (2020) to identify developing larva in terms of both performance (96.2%, 80.4%) and speed (Gungormus 2020).

One of the most important performance criteria for a model that will work in real time is the detection time of the larvae in a photograph taken. In the test process, the average detection time of the larvae in all photographs was determined as 2.75 sec.

3.2. Detection of honeycomb cells

Larva are located within the honeycomb cells at the base. However, larvae with ideal size are located at different points of the honeycomb based on the point where the queen bee laid the eggs (Figure 4). In order to transfer larvae with the larva transfer tool, it is necessary to place the mechanism at the correct point above the honeycomb and to lower the larva transfer tool to the correct point of the cell. Lowering the larva transfer tool at the wrong location may cause damage to the honeycomb or larva, in addition the damaging the device. For this, it is necessary to ensure detection of the central points in the honeycomb cells. Honeycomb cells have hexagonal shapes. However, when examined carefully, the shape is very close to a circle. Based on this, round shapes were placed on the cells using the Hough Circles technique and the center points of the circles and hence the cells could be identified (Söylemez 2012).

For detection of honeycomb cells, the Open CV image processing library was used. Firstly, photographs were converted to greyscale. Later 'Hough circles' were placed on the greyscale photographs. As a result of this process, the center points and radius values for many circular areas placed on the photographs according to the parameters were identified. In accordance with these values, circles were drawn in green color on the photograph with OpenCV and the image observed in Figure 10a was created. However, there were too many circles as can be seen from the figure. At this point, based on the information that the dimensions of the honeycomb cells were the same size, circles with radius above or below a certain value were deleted. As a result, the central points of all cells located in the photograph were identified and labelled and are presented in Figure 10b. The same process was used for detection of the center points of the queen cups and was successful.



a) Detection with circles on honeycomb image b) Circles at honeycomb cell dimensions

Figure 10- Cell detection

3.3 Mechanism and movement algorithm for larva transfer tool

Operating system software was developed to monitor and control the whole system. The pseudo algorithm explaining operation of the operating system is as follows:

1:	$N_{qc} = 22 \leftarrow Total\ number\ of\ Queen\ Cup$
2:	Place larva transfer tool at central point of cell
3:	Take photograph of cell
4:	Identify larva with suitable size
5:	Identify cells
6:	Number larva with suitable size within cells
7:	$larvae = 1$
8:	**repeat**
9:	move to cell containing larva
10:	Take larva
11:	Move to queen cup
12:	Drop larva
13:	$larvae \leftarrow larvae + 1$

14:	**until** $larvae > N_{qc}$
15:	Finish

When the software is first operated, the system is moved to the center point of the honeycomb because queen bees generally lay in spiral form beginning from the center point of the honeycomb. Then the photograph of the honeycomb is taken and larva with ideal dimensions and the center of the cells are determined. Larva with ideal dimensions located in the honeycomb cells are numbered. The identified and numbered larvae are presented in Figure 11.

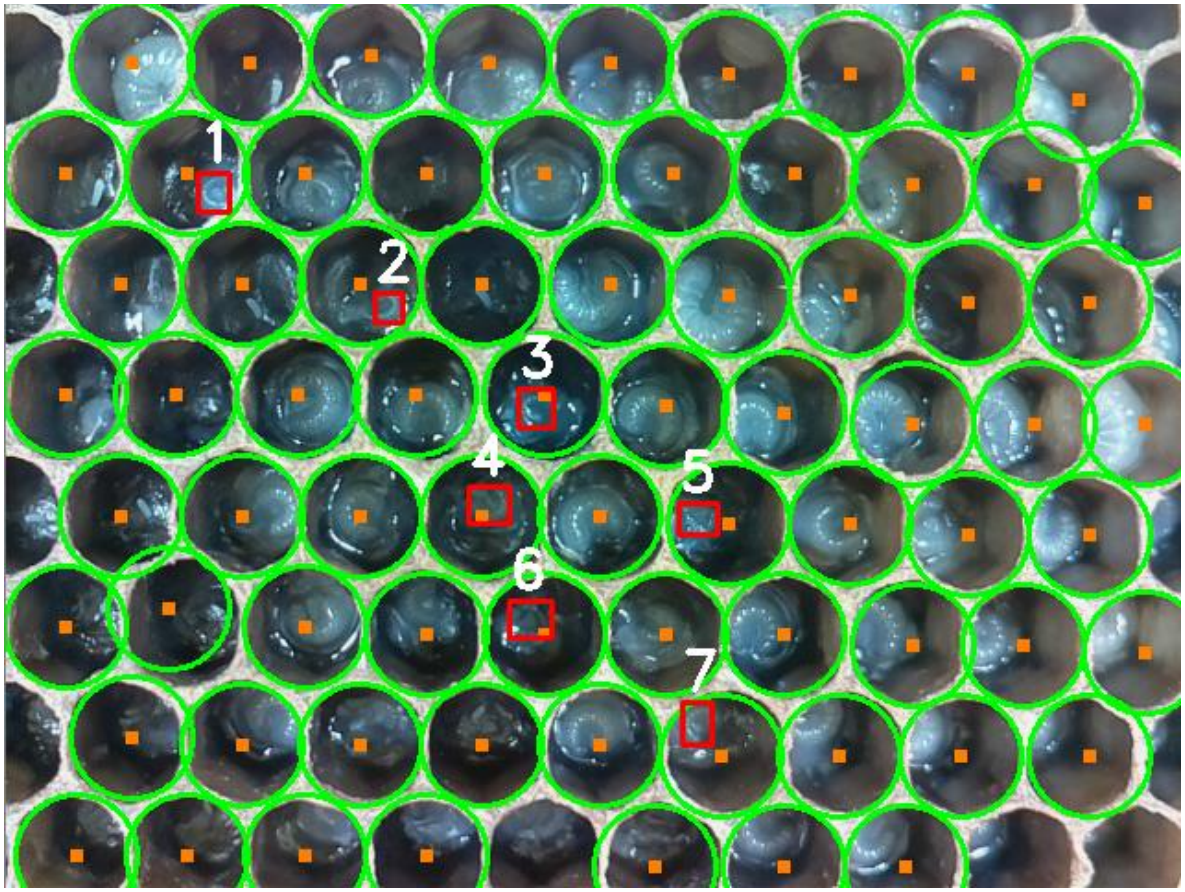
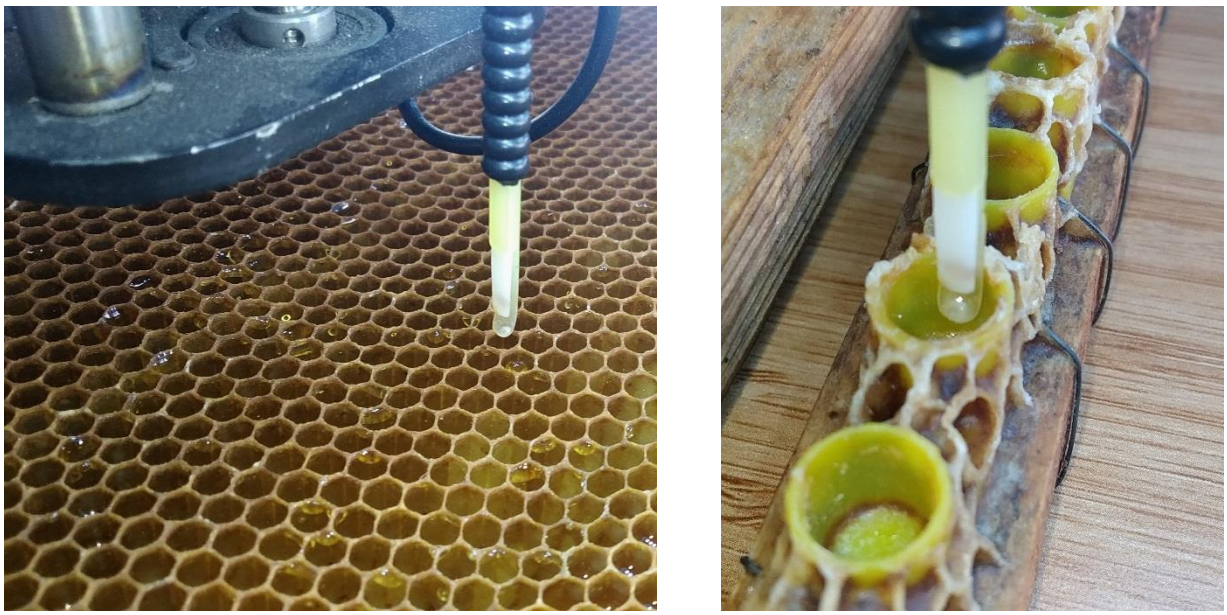


Figure 11- Detection and numbering of larvae

After numbering the larvae, the system begins the transfer process for all larva in order. For this, firstly the larva transfer tool moves to a point between the edge of the cell and the larva. Then the larva transfer tool is lowered 4 cm downward and moved toward the larva to ensure the larva is placed on the transfer tool tip. After taking the larva, the larva transfer tool is moved upwards by 4 cm and then moved to the point where the queen cups are located. The rod containing the queen cups and the cups have standard size and are continuously at the same point, so the system moves to the next cup in order. Then the tool is lowered 3 cm, the button on the transfer tool is pressed and the larva is dropped. The image taken after removing the larva is shown in Figure 12a, and the image for the larva being dropped is presented in Figure 12b.



a) Retrieving larva from honeycomb cells b) Leaving larva in queen cup

Figure 12- Larva transfer process

After the end of the larva transfer process, the same procedure is repeated until the number of queen cups is reached. After filling all cups, the operator simply needs to place a new rod and restart the system.

4. Results

The developed larva transfer tool was prepared to complete the larva transfer process automatically. Then it was tested with 5 dark and 5 light honeycombs. The tested honeycombs contained mean 400 larva with ideal size, with 200 on each side of the honeycomb (these values vary according to season, work by the queen, duration of use of honeycombs). After photographing the ideal size larvae, detection with the MobileDet+SSDLite model took mean 2.75 s. The same process lasted nearly 7 s with Faster R-CNN.

The larva transfer system successfully identified 95.56% of larvae in the 10 honeycombs. Attempts were made to transfer 440 of the identified larvae and 217 (50%) were successfully transferred. At this point, no difference was revealed in performance between dark- and light-colored honeycombs. Images of a successful transfer are presented in Figure 11. Additionally, it was determined that the mean duration for successful transfer processes was 20 s from when larvae were identified. A normal person has mean larva transfer rate of 7 s (this was the mean rate for workers in the apiary where the study was performed).

5. Conclusions

There was no study in the literature about the detection and autonomic transfer of bee larvae with appropriate size for royal jelly production. This study used a trained convolutional artificial neural network using Mobiledet+SSDLite to achieve larva detection with high performance. The larva transfer mechanism developed transferred the identified larva with nearly 50% performance. The basic cause of unsuccessful transfer attempts was the variability in the depths of the honeycomb cells. Some larva did not adhere to the larva transfer tool and were pushed to the edge of the cell and could not be retrieved. Additionally, some honeycomb cells were morphologically deformed which caused errors.

Future studies to increase the performance of the system:

- An end effector (perhaps with vacuum) design may be used to pay attention to variations in depths of the honeycomb cells and to retrieve the larvae from the cells without damage.
- Faster embedded system (FPGA-supported systems etc.) and mechanical equipment may be used to increase the transfer rate.
- The rods of queen cups used in the apiary studied contain 22 cups, but there are designs containing 30 or 60 cups and with different shapes. An algorithm may be developed which calculates the number of cups and operates accordingly.

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