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# Evaluation the UV sterilization of *Paenibacillus larvae* on beehive building materials

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**ABSTRACT**

This study presents the possibility of killing almost all microorganisms such as fungi, bacteria, spore forms, and viruses by sterilization process. European foulbrood (EFB) and American foulbrood (AFB) is a highly infectious bacterial honeybee disease caused by *Melissococcus plutonius* and *Paenibacillus larvae*, respectively. Removal of spores from contaminated beehives is a critical factor in controlling EFB and AFB. The purpose of this study was to evaluate the effectiveness of ultraviolet (UV) in killing *Paenibacillus larvae* spores on PVC, and wood hives. Hives infected with *Paenibacillus larvae* spores were treated with two UV powers (6 and 8 W) for up to 15 min. Sterilization at 8 W for 15 min resulted in a more than 6.6 log reduction in the number of *Paenibacillus larvae* spores on the PVC hives. Under the same experimental conditions, the reduction in wood hives was 6.2 log. Reductions achieved in *Paenibacillus larvae* spores on PVC hives after 5, 10 and 15 min of sterilization were significantly ($p<0.05$) higher than those on wood hives. So it is recommended to sterilize hives contaminated with spores with UV lamps.

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## 1. Introduction

Strong honey bee (*Apis mellifera*) colonies are needed for more production and effective pollination, but there is a relationship between disease factors and colony strength. Colonies are infected with fungi, viruses, and bacteria, such as European foulbrood (EFB) and American foulbrood (AFB), which is caused by spore-forming bacteria *Melissococcus plutonius* and *Paenibacillus larvae*, respectively (Alonso-Salces et al., 2017). If there is no cure for the disease, the spores will spread and infect the larvae, whereas spores do not cause infection in adult bees (Arredondo et al., 2017). AFB-affected larvae decompose to a glue-like colloid liquid, producing a specific smell (Fries et al., 2006). So, it causes economic loss to beekeepers. As it can live in colonies for many years, spreading via combs and honey products (Teixeira et al., 2018).

New Zealand legislation specifies that all bees, bee products, and appliances associated with AFB diseased colony must be burnt (Matheson and Reid, 2018). Generally, there are several methods of sterilizing beehives, such as flaming or scorching with a blowlamp (Gajger and Tomljanović, 2013) infrared; hand held electric paint stripper or immersion into molten paraffin (Del Hoyo et al., 1998); chemical sterilization with disinfectants or acetic acid; boiling in hot water or caustic soda (sodium hydroxide); steaming with hot air; Ozonation (Patil, 2014; Emrah and Kursat, 2018); low pressure plasma (Priehn et al., 2016); methyl oxide; and gamma radiation (De Guzman, 2011).

Options for sterilization available for PVC hives are limited compared to the range of treatments available for wooden hives (Fera, 2013). Del Hoyo et al. (1998) found that immersing wooden frames in molten paraffin was a very effective sterilization method, but handling of molten paraffin requires special equipment and protective clothing, which the small beekeeper does not have (Fera, 2013). *Paenibacillus larvae* spores are resistant to a wide variety of treatments such as heat, desiccation, and chemicals (Genersch, 2017). Dobbelaere et al. (2001) reported that the whole elimination of *Paenibacillus larvae* spores on wooden hives could be completed when heat and chemical disinfectants were used at high concentrations. They also reported that the scorching was not acceptable as it was effective against *Paenibacillus larvae* spores at the surface of the material. These disadvantages of the traditional sterilization methods for hive materials led to the use of alternative methods such methyl oxide, low pressure plasma, and gaseous ozone. However, these methods are expensive and available only to beekeepers who work near a treatment facility (James, 2011), so sterilization by ultraviolet (UV) is an effective process for killing spores. Ultraviolet (UV) is an electromagnetic wavelength that microorganisms absorb most of its energy resulting in a germicidal effect [photochemical reaction alters essential molecular components (DNA and RNA)]. Sterilization by ultraviolet (UV) dependent on duration and intensity (Newman and Bond, 2004). Also, the inactivation of viruses, protozoa, and bacterial pathogens by UV radiation has been stated by Chatzisymeon et al. (2011). They reported that UV radiation

aids oxidation to occur, which is known as photolysis processes and results in bond cleavage of organic molecules. Stewart-Wade (2011) stated also that, the UV light treatment is a chemical-free method which, uses exposure to a specific wavelength at a specific bulb power to inactivate microorganisms. UV light has radiation with a short wavelengths than visible light, and therefore has higher energy. UV radiation at 254 nm is the most common wavelength used for killing pathogens. Ultraviolet does not require storage, special handing or mixing considerations like chemical sanitizers. The PVC, and wood are the most common materials for constructing beehives, so the aim of this research was to find and evaluate a safe, effective, environmentally friendly and powerful sterilization method for all types of hives.

## 2. Materials and methods

### *2.1. Sample preparation*

The source of spores forming bacteria *Paenibacillus larvae* caused by AFB was obtained from an infected apiary located 31º 10' 13" N, 31 47' 56" E at Meet-Salseel city, El-Dakahlia Governorate, Egypt. The spores of *Paenibacillus larvae* were used to contaminate the studied hive materials. Isolation, cultivation and bactericidal concentrations of AFB spores were carried out according to the method described by De Graaf et al. (2013). Cultures of *Paenibacillus larvae* were put in Petri dishes filled with agar and incubated at 35°C most of the cells speculated. Spore suspensions were achieved by transferring colonies from Petri dishes into distilled water and pooled. The suspension was centrifuged and suspended in distilled water. The spore concentration of the suspension was determined by plate count and adjusted to 107 spores/mL with distilled water. The suspension was saved in the refrigerator until usage. It was heated in a water base to activate spores before inoculation of the studied hive materials (Torlak and Isik, 2018). Pieces of hive materials (PVC, and wood) with dimensions of 5×4×2.5 cm were used. About 10 mL of the pooled spore suspension was sprayed on pieces of hive materials and saved in the refrigerator until Ultraviolet (UV) treatment.

### *2.2. Ultraviolet (UV) sterilization chamber*

Two UV-C Lamps (Figure 1) with a wave length of 254 nm were used as a source of UV light. Its specifications are listed in Table 1.

**Table 1.** UV-C lamp specifications

| | |
|---|---|
| Model | PHILIPS TUV 8W T5 |
| Certification | CE, RoHS |
| Overall Length | 302.5 (max) mm |
| Color temperature | Blue |
| Diameter | 16 mm |
| Lamp current | 0.15 A |
| Lamp voltage | 56-265 V |
| Max. wattage | 8 W |
| Average life (hrs) | 9000 |

Pieces of hive materials (PVC, and wood) were placed in the sterilization chamber with the contaminate surface facing up and 45 cm away from the UV-C lamp. Each group of treatment included three pieces of each type of hive material. Contaminated pieces were subjected to two UV powers (6 and 8 W), which were valued by using the merged rheostat. Sterilization was performed three times (5, 10 and 15 min) at an ambient temperature of 26°C.

**Figure 1.** Ultraviolet sterilization chamber

### *2.3. Counting of Paenibacillus larvae*

The number of *Paenibacillus larvae* on contaminated pieces before and after sterilization was recorded by colony counting technique, and the results were recorded as log cfu/piece.

### *2.4. Statistical analysis*

Data were edited in MS Excel (Microsoft Corporation, Redmond, WA, USA). The Levene and Shapiro-Wilk tests were conducted in order to check for normality and homogeneity of variance (Razali and Wah, 2011). The data were statistically analyzed using the Costat Program (Oida, 1997) to determine the significant effect of the mentioned variables on the study based on the probability ($P<0.05$). The experiments were carried out three times in all. All graphs were drawn using Microsoft Excel 2016.

## 3. Results and discussion

Decreasing in mean count numbers of *Paenibacillus larvae* on sterilized PVC, and wood pieces after 5, 10, and 15 minutes and at UV power of 6 and 8 w are shown in Figures 2, and 3, respectively. The primary mean count numbers on PVC, and wood pieces were recorded as 6.8±0.2, and 6.6±0.3 log cfu/pieces, respectively. After 15 min of UV sterilization, spore count numbers on wood spices were significantly decreased ($p<0.05$) by 4.6 and 6.2 log cfu/pieces at UV power of 6 and 8 w, respectively. These decreases were nearly recorded on PVC pieces after only 10 minutes of UV sterilization. Decreases of 6.3 and 6.6 log cfu/pieces were recorded on PVC pieces after 15 min at UV power of 6 and 8 w, respectively. The results mentioned that decreases in count numbers of *Paenibacillus larvae* spores on PVC pieces after 10 and 15 min were significantly higher than those on wood pieces ($p<0.05$). This result may be attributed to the porous structure of wood. When materials have a porous surface, the spores can be embedded in cavities that prevent the interaction of UV Sterilizers with the spores, thus declining their potential for *Paenibacillus larvae* inactivation. As a result, the use of UV radiation has a significantly impact on surface porosity.

Ultraviolet radiation is an effective method for the inactivation of bacterial spores (Sanchez-Salas et al., 2017; Nyangaresi et al., 2019; Pendyala et al., 2019). In the present study, I used UV radiation to study the reduction in viability

of *Paenibacillus larvae* on hive building materials. However, it should be known that the UV effect on bacterial spores is dependent on duration and intensity (Newman and Bond, 2004). The effect of UV can be described by oxidative stress damage in spores (Taylor et al., 2020).

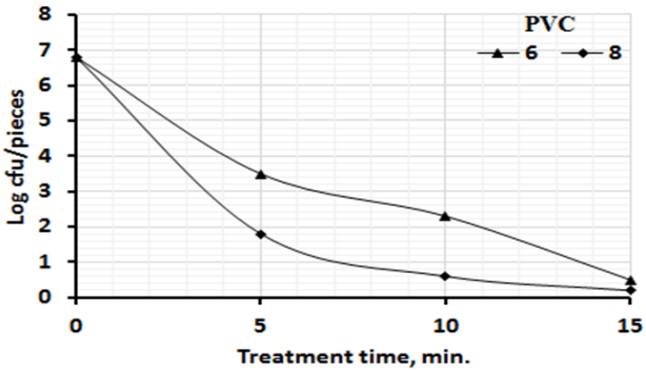


**Figure 2.** Population of *Paenibacillus larvae* on PVC pieces under different levels of UV power and treatment time

The best curve for the relationship between the population of *Paenibacillus larvae* "P" and treatment time "T" under both UV powers is the linear equation as shown in Equations (1 and 2).

At 6 w P = -0.402 T + 6.29 $R^2$ = 0.9561 → (1)
At 8 w P = -0.420 T + 5.50 $R^2$ = 0.7935 → (2)

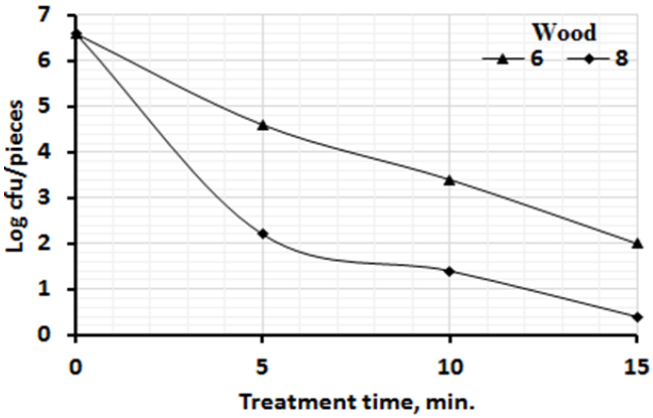


**Figure 3.** Population of *Paenibacillus larvae* on wood pieces under different levels of UV power and treatment time

At 6 w P = -0.300T + 6.40 $R^2$ = 0.9877 → (3)
At 8 w P = -0.388T + 5.56 $R^2$ = 0.8390 → (4)

Results in Table 2 indicated that the mean count numbers of *Paenibacillus larvae* decreased with beehive building materials, UV power and treatment time, according to the descending order (wood > PVC), (6 w >8 w) and (0 >5 > 10 > 15 min), respectively. Also, the building materials had a highly significant effect on the mean count numbers of *Paenibacillus larvae*. Minimum estimate is observed with PVC, respectively. Moreover, the different levels of UV power and treatment time affected the mean count numbers of *Paenibacillus larvae* significantly. Higher and lower estimates are shown at 6 and 8 w, respectively. Samples without treatment were coupled with the highest estimates compared to treatment times of 15 min which were coupled with the lowest estimates.

**Table 2.** Mean count numbers of *Paenibacillus larvae* affected by studied factors

| Factors | | Population, log cfu/pieces |
|---|---|---|
| Building materials | PVC | 2.8±0.11a |
| | Wood | 3.4±0.15b |
| | P-value | <0.0001 |
| UV power | 6 w | 4.0±0.24a |
| | 8w | 2.9±0.12b |
| | P-value | <0. 0001 |
| Treatment time | 0 min. | 6.7±0.31c |
| | 5 min. | 3.6±0.16a |
| | 10 min. | 2.3±0.11b |
| | 15 min. | 1.1±0.07d |
| | P-value | <0. 0001 |

## 4. Conclusion

This study investigated the inactivation performance of UV radiation toward *Paenibacillus larvae* causing AFB. Two building materials were studied under two UV power levels and three duration times. The minimum population of *Paenibacillus larvae* was obtained for the studied building materials after 15 min and at maximum UV power of 8 w. The reduction in the population of *Paenibacillus larvae* spores on PVC pieces was significantly higher than those on wood pieces. So, it is recommended to use UV radiation in the sterilization of beehive building materials, especially for PVC or plastic hives. Complete removal of *Paenibacillus larvae* that causes AFB from beehive building materials is an important requirement to avoid spread of American and foulbrood diseases. So, more studies are still necessary to achieve a more significant decrease in the population of *Paenibacillus larvae* on building hive materials.

**Compliance with Ethical Standards**

**Conflict of Interest**

The author declare that he has no conflict of interest.

**Authors' Contributions**

**Mohamed Ali Ibrahim Al-Rajhi**: Validation, writing-original draft, methodology, investigation, conceptualization, formal analysis, data curation.

**Ethical approval**

Not applicable.

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**Data availability**

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**Consent for publication**

Not applicable.

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