Sterilising beekeeping equipment infected with American Foulbrood Disease spores

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Beekeepers use a wide variety of products to clean and sterilise gloves and other equipment that have come in contact with a colony infected with American foulbrood disease. These are used to either kill spores of *Bacillus larvae* (the causative agent of American foulbrood disease) and/or to physically remove them.

The aim of this study was to test the ability of a range of products to kill *B. larvae* spores. We asked beekeepers which substances they used to sterilise equipment. The most common were sodium hypochlorite, methylated spirits, Dettol® and Savlon®. We tested these products along with ethanol and Virkon®. It must be noted, however, that under the new Pest Management Strategy for American Foulbrood disease, any method used to sterilise equipment contaminated with *B. larvae* spores needs first to be approved by the Management Agency of the Strategy (National Beekeepers Association).

Sodium hypochlorite

Sodium hypochlorite is a commonly used sterilising agent. It is the active ingredient in Janola®, which contains about 3% sodium hypochlorite. *B. larvae* spores were added to sodium hypochlorite solutions of various concentrations (0, 0.5, 1, 1.5, 2, 2.5 and 3% a.i.) and left for a range of times (0, 30, 60, 90, 120, 150, 180, 360 min and 24h). Two samples of 5 mls of each suspension were passed through a Millipore filter to remove the spores. The filters were then flushed with sterile water to remove any remaining hypochlorite, and placed on growth media to determine spores viability. The number of colonies growing was counted and averaged for each suspension.

No *B. larvae* colonies grew on any of the filters other than the control (0% hypochlorite, indicating that the lowest concentration (0.5%) and shortest time (30 min) tested was sufficient to deactivate spores. The trial was repeated with lower hypochlorite concentrations (0, 0.1, 0.2, 0.3, and 0.4%) and shorter times (30 sec, 10, 20, 30 40 and 50 min).

The time required to prevent colony growth decreased with increasing hypochlorite concentrations (Fig. 1). Short exposure to hypochlorite (less than 1 minute) increased spore germination.

Concentrations of 0.3-0.4% hypochlorite deactivated *B. larvae* spores in 20 min. However, to ensure a safety margin it is probably better not to use concentrations less than 0.5%. Care needs to be taken with the types of materials being treated. Some plastics and metals may degrade in hypochlorite solutions. You should run a small trial first to ensure the hypochlorite will not dissolve what you want to sterilise. It is not possible to predict how frequently the hypochlorite solution should be changed. This will depend on how clean the equipment you are sterilising is, how much equipment you are sterilising and what is being sterilised. If you were sterilising large amounts of equipment it would be best to use higher concentrations of hypochlorite and longer times than that recommended above. Hypochlorite needs to be kept in the dark as it is broken down by sunlight and should be replaced after use.

	-Spores		+Spores	
	Average	S.E.	Average	S.E.
Water	64.75	17.23	52	4.38
Dettol	52.67	16.94	44.33	9.6
Virkon	0	0	24.88	1.36
Savlon	63.17	17.69	79.33	13.87

Table 1: Average number of *B. larvae* colonies per plate (average) and standard errors (S.E.) for water, Dettol®, Virkon® and Savlon® both without (-spores) and with (+ spores) additional *B. larvae* spores added after centrifuging.

Ethanol and Methylated spirits

Spore suspensions were added to either drum ethanol (98%) or methylated spirits (100%) and the spores tested for viability after 10 min and 24h using the methods described for the sodium hypochlorite. Colony growth still occurred (>100 colonies/plate) after the spores had been in either ethanol or methylated spirits for 24h, indicating that neither are effective at deactivating *B. larvae* spores.

Savlon®, Dettol® and Virkon®

B. larvae spores were added to Savlon® (90%), Dettol® (90%), and Virkon® (90%). After 1 hour, each of the suspensions was centrifuged for 1h, and the spores resuspended in sterile water. This was repeated twice to remove the Savlon®, Dettol®, and Virkon®. The spores were then resuspended in water and each sample split into two. One half was spread onto growth media while the other half had further viable *B. larvae* spores added and spread on growth media and incubated for 3 days to determine if there was any of the original solutions present that could have inhibited colony growth.

Growth was recorded with Dettol® and Savlon® after the suspensions were centrifuged (Table 1) both with and without additional spores added. Growth was only recorded with Virkon® when additional spores were added. This indicates that Virkon® is effective at deactivating *B. larvae* spores while Savlon® and Dettol® are not. This needs to be retested to determine if Virkon® can deactivate spores at lower concentrations and exposure times.

Summary

Of the products tested only sodium hypochlorite and Virkon® were effective at deactivating

B. larvae spores. Some of the other products tested may, however, be useful in physically removing spores through washing if they are used in large quantities.

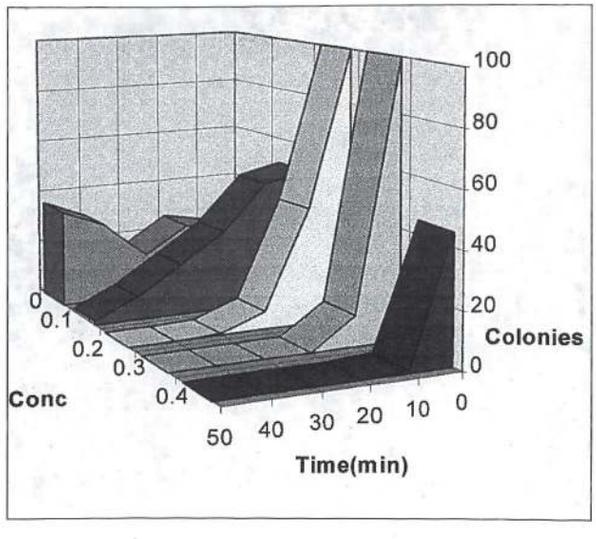

Fig 1: Average number of *B. larvae* colonies (colonies) per filter disc after the spores were in hypochlorite solutions of varying concentrations (%) for a range of times (Time).