Title: Determining Concentrations of Disinfectants for the Control of Poultry House Bed Bugs (*Cimex lectularius* L.)

Background and Significance: *Cimex lectularius* L., commonly known by the name bed bug is an insect in the order Hemiptera and family *Cimicidae*. Often bed bugs range from 4 millimeters to 6 millimeters in size and are reddish-brown in color. There are five nymph stages during an incomplete metamorphosis life cycle of a bed bug. Each nymph stage must take a blood meal before they molt to the next instar. Bed bugs are most commonly known as a human ectoparasites, but they also feed on bats and birds. Bed bugs have been reported in poultry houses since the 1900s (Szalanski 2018). Bed bugs are found in cage free egg production systems due to the natural cracks and crevices in housing equipment that make optimal hiding/harborage locations for them. Due to the fact that bed bugs are nocturnal, they are not often detectable during the day time in the barns, however feeding lesions can be seen on the legs and the breasts of the chickens (Szalanski 2018). Bed bugs in these cage free systems pose a safety concern for both the chickens and the employees of the farm. Bed bugs can bite the workers as well as hitchhike to their homes and other locations that they visit. Thus, leading to their spread. Bed bug inspections conducted by Ashbrook et al. (In Prep) showed that bed bug population numbers increased through the one year growing cycle.
Due to the persistent bed bug infestations in cage free poultry houses, the growers wanted to know if disinfectants such as industrial grade bleach and Virkon S that are used for microbial load reduction in chicken houses, would also be effective for the control of bed bugs. My research project was to determine the LC_{50} and LC_{90} (lethal concentrations that kill 50 and 90% bed bugs) of two disinfectants; industrial grade bleach and Virkon S. The industrial grade bleach has a Sodium hypochlorite concentration of 12.5%. Virkon S is made of Potassium peroxymonosulfate (21.41%), Sodium Chloride (1.50%), and other ingredients (77.099%). Both of these chemicals are effective in killing bacteria and viruses.

Materials and Methods:

Insects: Adult bed bugs (males: females in 1:1 ratio) from the Poultry House strain which was collected in 2015 from the state of Tennessee were used for all bioassays. This strain of bed bugs was adapted to laboratory rearing. They were fed weekly on defibrinated rabbit blood (Hemostat Laboratories) using the Parafilm® membrane feeding method (Chin-Heady et al. 2013). Environmental conditions used for rearing and conducting bioassays were 25°C temperature, 40% relative humidity and 12:12 h light: dark cycle.

Preparation of Solutions: Desired dilutions of industrial grade bleach, Virkon S were prepared in distilled water. Concentrations that were used are shown in Table 1.

Table 1. Concentrations of industrial grade bleach and Virkon S used for direct spray bioassays

Chemical	Industrial Grade Bleach	Virkon S
1	6.25%	40%
2	4.166%	20%
3	3.125%	10%
4	1.5625%	5%
5	0.78125%	2%
6	0.625%	1%
7	0.3125%	0.5%
8	0%	0.25%
9	-	0%

Treatment of bed bugs with disinfectants and mortality observations: Once the concentrations for the disinfectants were prepared, they were poured into spray bottles (3.3 oz or 100 ml; Walmart, Bentonville, AR) and sprayed directly to approximately 10 adult bed bugs (males and females in 1:1 ratio) (Fig. 1). Around 0.318ml of spray solution was used per Petri dish. This volume was equivalent to the application rate of 1 gallon per 1000 sq. ft. Spray treatments were conducted in a fume hood (Fig. 2). The treated bed bugs were set aside for 5 minutes and then transferred into smaller untreated Petri dishes with filter paper substrate. Controls were sprayed with ~0.318 ml of distilled water. At least 3–4 replicates were performed for each concentration of bleach or Virkon S. As stated before, each replicate consisted of 6 to 10 adult bed bugs collected from the Poultry House strain. Mortality was scored every 24 h after treatment up to 72 h. However, only the 72 h data was used for further analysis.

Figure 1. Photos showing spray bottles and Petri dishes/ bed bugs used for bioassays

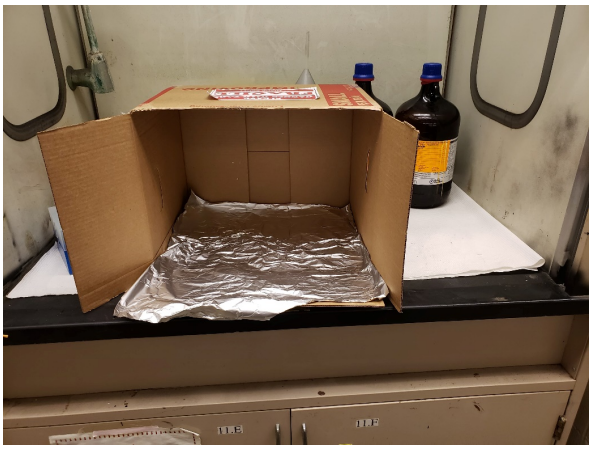

Figure 2. All spray treatments were conducted in a cardboard box placed inside a fume hood.

Data analysis: To determine lethal concentration estimates that cause 50 or 90% bed bug mortality (LC_{50} or LC_{90}) probit analysis was performed with raw concentration-mortality data using the SAS 9.4 software.

Results:

Lethal concentration estimates for Virkon S: The Virkon S LC_{50} was 0.21% and LC_{90} was 1.08% (Table 2). At the label application concentration or dilution of 1%, Virkon S may be lethal to 90% of the population that is directly sprayed with this chemical. The p-value obtained through probit analysis was greater than 0.05%, which indicates that the mortality response of the Poultry House strain bed bugs to Virkon S was homogeneous. The plot of concentrations of Virkon S versus percent mortality at 72 h was somewhat linear (Fig. 3). Average mortality in water-treated control bed bugs was less 5%. Probit analysis corrected for control mortality while determining LC estimates.

Table 2. Lethal concentration estimates for Virkon S and Bleach as determined by probit analysis (at 72 h after treatment)

Treatment	N	LC50 (%) [Fiducial Limits]	LC90 (%) [Fiducial Limits]	Slope ±SE	X^2	d.f.	P-value
Bleach	297	**3.36** [LL: 2.32] [UL: 4.49]	**4.18** [LL: 3.64] [UL: 70.08]	13.57± 4.91	13.97	5	0.016
Virkon S	255	**0.21** [LL: 0.12918] [UL: 0.27640]	**1.08** [LL: 0.78] [UL: 1.83]	1.78 ± 0.29	2.82	4	0.59

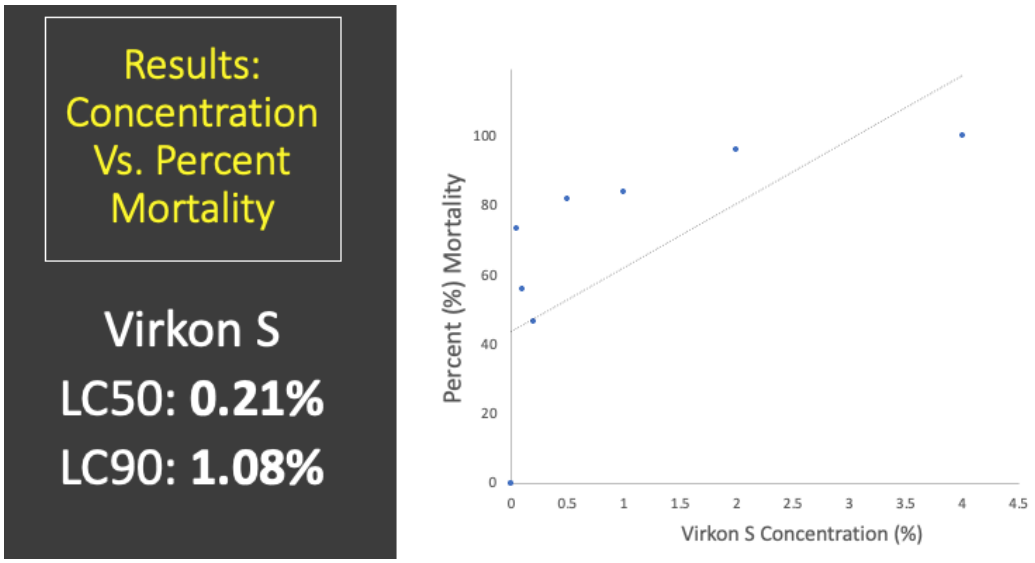

Figure 3. Concentration-mortality graph for Virkon-S at 72 h after treatment

Lethal concentration estimates for industrial grade bleach:

For industrial grade bleach the LC_{50} was 3.36% and the LC_{90} was 4.18%. For bleach the concentration-mortality graph was not linear (Fig. 4). Also, the p-value of <0.05% suggested that the adult bed bug mortality response to various concentrations of bleach was heterogeneous. Mortality in the control (deionized water) treatment was less than 5% and it was accounted while conducting probit analysis.

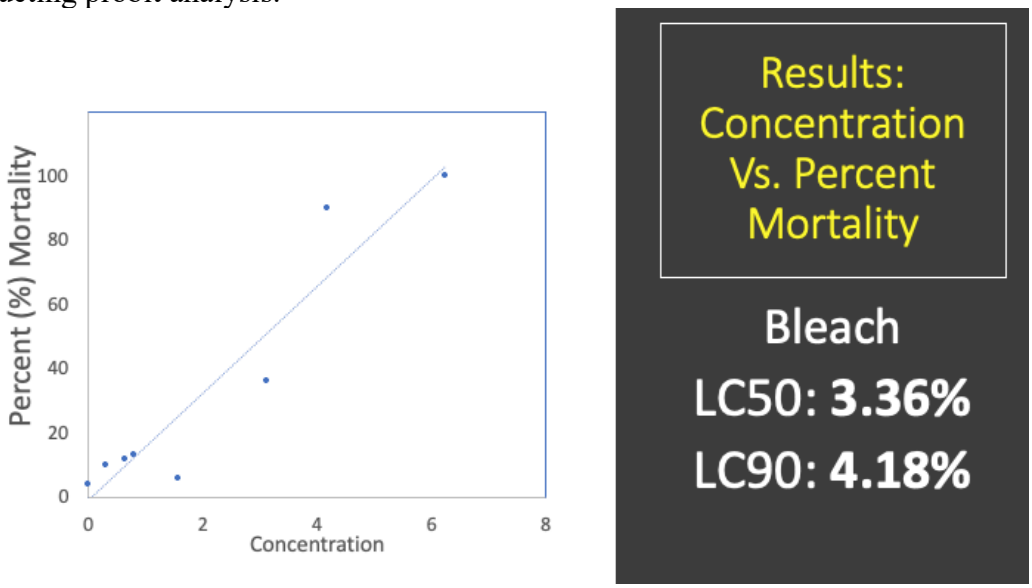

Figure 4. Concentration-mortality graph for industrial grade bleach at 72 h after treatment

Discussion:
This was not a product efficacy comparison project, meaning that one disinfectant was not compared for its efficacy against bed bugs in relation to the other. However, a downside to using bleach is that during the winter months it can lead to the built up of ammonia and the explosive chemical hydrazine in poultry barns that hold up to 45,000–50,000 egg laying chickens. This happens due to reduced ventilation in the poultry houses during the winter season. Therefore, Virkon S is a better disinfectant and thereby a better bed bug killing agent for use during the winter months. Although effective and economical, disinfectants such as bleach and Virkon S should not be solely relied on for bed bug control in poultry barns. Whenever possible and safe, insecticides could be used in combination with disinfectants to reduce bed bug population numbers. Since the workers already spray the disinfectants to kill the microbes in poultry barns, the use of disinfectants along with insecticides represents an economically efficient means of bed bug control.

My Experience:
Overall, there were some minor difficulties that I faced in implementing this project. One of the difficulties was related to get the precise volume of 0.318ml chemical solution sprayed over bed bugs. Each spray bottle did not have the same accuracy as the previous one. Also, rearing enough bed bugs was more difficult than I initially thought. I had to adopt a strict schedule to feed and sort out large nymphs to obtain adult bed bugs of specific age. It was also discouraging to see data not turn out how it was anticipated. However, in working with live animals some inherent variability in their response is expected. The next steps for this project are to test the LC_{50} and LC_{90} concentrations of Virkon S and bleach against the nymph and egg stages of poultry house bed bugs.

Reference:
Doggett S. L., Miller D. M. & Lee C. Y. (2018) “Advances in the Biology and Management of Modern Bed Bugs”. Wiley Blackwell.
Specifically: Chapter 40, *Poultry Industry*, written with Allen Szalanski.

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