POULTRY PESTS AND PESTICIDES

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Publication Date
2026-06-15

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A capstone project submitted for Graduation with University Honors

May 08, 2026

University Honors

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Abstract

In recent years bed bugs have become a pest of interest due to their increasing populations. This, and bed bugs' increased pesticide resistance, requires development of new control strategies. Insect pathogenic fungi, specifically *Beauveria bassiana* and essential oils like citronella are both promising biopesticide products that could prove effective and environmentally friendly. Experiments using biopesticides in the past have shown that the environment they are used in can have a significant impact on how effective they are against the chosen insect. So we used laboratory bioassays with *B. bassiana* and citronella oil products to evaluate their efficacy against bed bugs in different environments. In these bioassays, we found that of the two commercial products used, Naturganic did have a statistically significant impact on the bed bug mortality, and BotaniGard did not.

Acknowledgements

I want to sincerely thank my faculty mentor, Dr. Amy Murillo for the role she played in this research project. It was my honor and privilege to work with someone who was as excited about this project as I was. I will forever appreciate the guiding role she played throughout this whole process, and her ability to give me the kind of support I needed at any moment throughout my project. I cannot thank her enough for the time and energy she put towards this project and my success as a researcher. It was a joy and honor to work with her and be a part of her lab, one that I will carry with me into the rest of my career as an entomologist.

Introduction

Bed bugs (Hemiptera: Cimicidae) are small, wingless insects classified as hematophagous ectoparasites (external parasites that feed on blood). It is believed that bed bugs evolved in caves within the Mediterranean and Middle Eastern regions where they were nest parasites of bats and birds (R.L. Usinger, 1966). Although the exact time period is unknown, ancestral bed bugs most likely evolved approximately 145-65 million years ago, and it is believed that humans became the hosts of bed bugs when they started to inhabit caves sometime during the Pleistocene, Paleolithic, and Neolithic periods (R.L. Usinger, 1966). Bed bugs are one of human's longest associated pests with the earliest records of them living with humans from 400 B.C. Greece (Koganemaru and Miller, 2013). Bed bugs feed when their chosen hosts are asleep, and can live for weeks or months without feeding. The common bed bug, *Cimex lectularius,* typically feeds on humans, however, they will opportunistically feed on other animals such as rodents and birds. It is thought that due to the close association with humans, bed bugs were introduced to poultry farms as early as 1914, with farmers reporting infestations of them (Mote, 1917). Knowing this history, it is no surprise at all that they have come to be associated as a pest for poultry.

There is nothing to indicate that bed bugs transmit any pathogens that cause human diseases, but their bites can cause allergic reactions on the skin, or an irritation rash (Koganemaru and Miller, 2013). Bed bug infestations on chickens can lead to several symptoms such as overgrooming, anemia, restlessness, and an overall decrease in the health of the poultry (Gonzalez-Morales et al., 2022). These infestations were largely eradicated with the use of newly invented pesticides, namely DDT (dichlorodiphenyltrichloroethane) which they began using in the 1940s. However, due to its negative environmental and health effects, it is now banned in most countries. Pyrethroid-based insecticides are now the most commonly used to control bed bug populations. However, because it is so commonly used against them, Pyrethroid resistance is present in many bed bug populations across the world (Davies et al., 2012). Target-site resistance (knockdown resistance [kdr] mutations) have dramatically increased in bed bug populations in the last decade (Gonzalez-Morales et al., 2022).

A resurgence of bed bugs has been reported in more than 50 nations, including all regions of the globe, and it is now evident that the evolution of insecticide resistance in bed bugs has played a significant role (Dang et al., 2017). There have been at least 12 classes of insecticides evaluated or used, including organophosphates, carbamates, pyrethroids, neonicotinoids, pyrroles, insect growth regulators (IGRs), botanical insecticides, as well as inorganic silica and mineral compounds (Doggett and Lee, 2023). Pyrethroids, insect growth regulators (IGRs), and pyroles are the only pesticides that are still permitted to be used indoors for treating infestations now. There are currently 16 pyrethroid insecticides and 5 pyrethrin insecticides that are available to treat bed bugs in the United States, some of which are over the counter products available to the general public. There is a lot of variation in the effectiveness of these pesticides between different bed bug populations, insecticide formulations, and specific pyrethroids and products (Koganemaru & Miller, 2013). Public opinion of pesticides is also generally poor because of the harm they have caused to the environment.

Since bed bugs are reemerging as an identified public health pest of concern, it is important to consider their dispersal, and potential avenues that lead to human infestation. The way that bed bugs most commonly disperse is passive dispersal, where they can easily adapt to different hosts and will move from host to host with little trouble when humans are not accessible (Steelman et al., 2008). This process creates a potential avenue of reinfestation from poultry to humans. Due to the growing bed bug populations with pesticide resistance, highly pesticide resistant bed bugs are expected to be introduced into poultry farms (Gonzalez-Morales et al., 2022). This is why previous control methods need to be examined, and new ones introduced.

Xenointoxication (the systemic treatment of hosts to kill parasites) has been widely used for both endoparasites and ectoparasites. It is a very common avenue used when treating animals for pests. Ivermectin is an antiparasitic drug that has been commonly used for humans, companion animals (dogs and cats), and livestock (cattle, horses, sheep, and swine). Xenointoxication has been used to control bed bugs that acted as ectoparasites towards chickens. Research found that ivermectin may not be effective for eliminating bed bugs (Cirak et al., 2018). Fluralaner (an isoxazoline, IRAC group 30), was effective at killing bed bugs after a single blood meal from a medicated chicken, even 28 days after the chickens were treated (Gonzalez-Morales et al., 2022). All of the field collected bed bug strains showed a high resistance to deltamethrin (a pyrethroid commonly used to manage bed bug infestations), but bed bugs from these strains were highly susceptible to fluralaner (Gonzalez-Morales et al., 2022). Bed bugs may also develop resistance to this novel pesticide as well. The best solution is to use varied pesticides to prevent resistance from building. These could prove to be very effective chemical methods against bed bugs, but chemical methods may not be desirable for some members of the public. Biopesticides are a viable avenue to treat pest problems, or supplement other pesticides.

Control methods that don’t employ chemical use have increased in popularity to reduce environmental harm and combat the effects of current pesticide resistance. One such method has been biological agents, or biopesticides, which are derived from natural sources. A biopesticide that is currently promising is an entomopathogenic fungus, *Beauveria bassiana*, which causes disease in insects (Anderson et al., 2011). It has proven to be an effective biopesticide against bed bugs in the past, but has yet to be used as a way to control poultry bed bugs. Another biopesticide that has been used successfully has been pesticides using citronella (*Cymbopogon nardus L.*) extract to control insects on crops (Telaumbanua et al., 2021; Istianto et al., 2025). Some dust formulations of inorganic insecticides are available for use in poultry environments, but these environments prove challenging when it comes to the efficacy, as it can provide inconsistent results (Gonzalez-Morales et al., 2022).

In previous experiments, it was shown that the substrate that the conidia of the *B. bassiana* was sprayed on, which affected the efficacy of the biopesticides's mortality rate. Comparing clay, wood, and cement substrates, clay was the most successful (Blanford et al., 2012). They tested these three different types of substrates against adult female *Anopheles stephensi* and *Anopheles gambiae* mosquitoes. They were focused on testing how well the fungus worked after being stored for a long period of time (over two years). Additionally, they tested the persistance of the spores on the mosquitoes after exposing them to the treated substrates for an hour, then monitored them for at most seven months. The experimental procedure of Blanford et al. (2012) will not be replicated here since the goals of the respective studies are different. But, these studies are still crucial because they show that the substrate biopesticides are used on will have a significant effect on insect mortality.

In Acharya et al. (2015), *B. bassiana* on several substrates successfully killed house flies. Bed bugs live in very different environments than flies and currently available research has not explored the efficacy of *B. bassiana* on the types of substrates that poultry bed bugs would occupy. *B. bassiana* has only been tested against bed bugs using materials emulating a human environment (e.g. cotton sheets or filter paper) (Aak et al., 2018), which poses the question of how effective it will be.

Citronella-based pesticides can significantly reduce the insect pest population (brown plant hoppers, green grasshoppers, stink bugs, leaf flies, and armyworms) on crops such as rice plants (Telaumbanua et al. 2021), as well as aphid populations on chili plants (Istianto et al. 2025). Plant derived essential oils have also been used against bed bugs, with mixed results. Citronellic acid was tested as a repellent against bed bugs, and although bed bugs were adverse to it, that did not stop them from seeking a blood meal and feeding (Gonzalez-Morales, Terán, and Romero, 2021). It was shown to not be an effective pesticide against bed bugs, but other essential oils were successful to some degree (Singh et al., 2014). There have been very mixed results from citronella based pesticides against insects in general, and poultry bed bugs and the environment that they reside in has not been tested at all. So it is important to test these pesticides in the unique environment to determine if they are effective.

Biopesticides such as diatomaceous earth, kaolin, sulfur, azadirachtin, and Beauveria bassiana have been tested in a poultry house environment (Mullens et al., 2012; Goddard and Maschek, 2015), but they were not nearly as effective as the chemical variety (45-46% success rate for the 2 chosen natural pesticides and 73-100% success rate for the 5 chosen ‘traditional’ or chemical products) (Goddard and Maschek, 2015). The selected pesticides in this case were a diatomaceous earth product and a plant oil product. If these biopesticides prove to be effective in killing bed bugs on different novel substrates, they may be viable for use in poultry houses. That is the goal of this experimental design. The different environments will have a range of effectiveness depending on how well the conidia takes to it, and how much coverage the environment could potentially provide to the bed bugs. It is more likely that an environment that provides high coverage, or an environment that does not appropriately host the conidia of the fungus would negatively influence the efficacy of the pesticides, which will cause mortality to be lower in those groups.

Materials and Methods

Insects

Bed bugs used in bioassays were from a Harold Harlan strain that was collected in Fort Dix, New Jersey in 1973 and has been maintained at North Carolina University since December 2008, and one part of it is now maintained in the Murillo lab colony. All experimental bed bugs were maintained in small plastic containers with mesh in the lid for ventilation and feeding. These containers have paper folded accordion style, feathers, and dirt/excrement used to provide shelter to the bed bugs. Insects were maintained in incubators kept at 70 degrees celsius on a 12 hour light: 12 hour dark cycle. Colonies were fed on chicken blood at most 7 days before the experiment and at least 1 day before experiment. Active adult bed bugs were chosen for each experiment. Bed bug rearing containers were placed in a larger metal container on a chill table and grabbed using soft grip forceps before being placed into the petri dishes. Each dish containing one bed bug. For Naturganic they were placed pre petri dish treatment, and for BotaniGard they were placed post petri dish treatment.

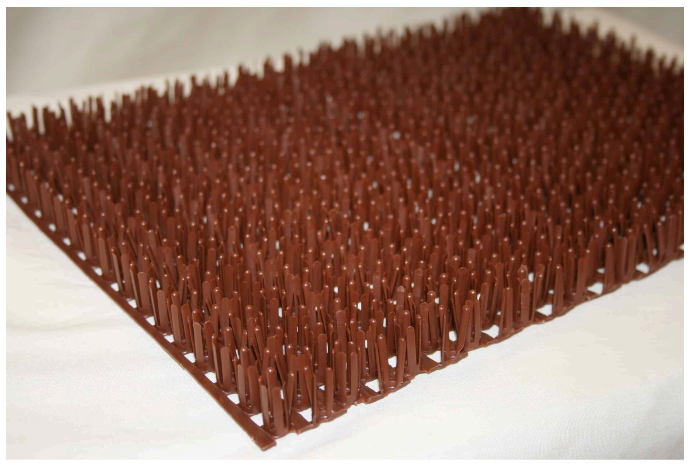
Products and Substrates

Bed bug control options are limited in poultry houses because of how sensitive poultry can be to various changes in and around their environment, this can affect their overall health, and the safety of their food products (eggs and meat). Many products most commonly used against bed bugs would be harmful to poultry in the concentrations needed to fight the bed bug pesticide resistance (Weeks et al., 2018). *Beauveria bassiana* is a fungus that will only affect insects negatively, so if it proves successful in an approximation of the environment that poultry live in, then it could very well be a completely viable alternative to what is currently being used. Additionally, citronella is safe to use around chickens, with the pest powder Naturganic being specifically advertised for use in chicken and swine production.

This study tested bed bug mortality after exposure to one of two different biopesticides, a commercially available form of *B. bassiana* called BotaniGard, and a citronella oil based pest powder called Naturganic. BotaniGard is advertised for use against insects in a greenhouse, field, or nursery crops with their commonly controlled pests including whiteflies, thrips, aphids, psyllids, mealybugs, beetles, plant bugs and weevils. BotaniGard has a high spore count and comes in a wettable powder form. Although it is not advertised for use against bed bugs it has been used successfully in other experiments against bed bugs (Aak et al. 2017). Naturganic is advertised as an eco-friendly pest powder for use against chicken and swine pests and claims that it is effective against bed bugs, cockroaches, or mites but there is currently no publicly available research on Naturganic pest powder, much less its efficacy on bed bugs. The website provides a data sheet claiming that there is 100% bed bug mortality 7 days after application. Naturganic is made up of 8% citronella oil, and 92% a mix of sodium bicarbonate, calcium silicate, and calcium carbonate.

Two different products were tested: BotaniGard 22WP (*Beauvaria bassiana* strain GHA, 2×10^{13} cfu/kg) as a water based-product and Naturganic (citronella oil) as a powder based product. BotaniGard was prepared according to the manufacturer's instructions by dissolving the product in water to dilute to the recommended concentration, 0.0024 g/mL and 2.5 mL per container. 0.155g of Naturganic was added per container. Both were applied to the manufacturer's specifications of product to surface area covered. In the Naturganic experiments the powder was applied directly to the substrate with bed bugs present. In the BotaniGard experiment the substrate was sprayed then dried for 48 hours before the bed bugs were added to the substrate to begin observation. There were two different substrates selected for use with these products. One was a plastic turf material that chickens often nest on in hen houses ('Turf' Figure 1B.), and the other was a mixture of straw, dirt, chicken feces, and feathers to simulate the floor of chicken enclosures ('Litter' Figure 1C).

A.



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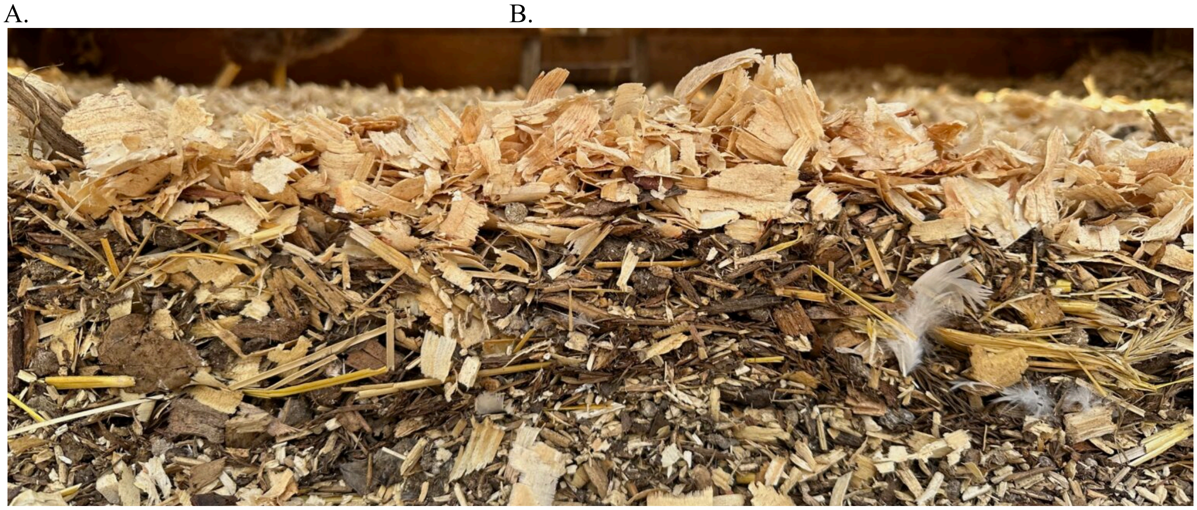

C.

Figure 1. A. Experimental design with petri dishes, Litter, and Turf. B. plastic Turf material. C. Chicken Litter made up of wood shavings, straw, dirt, feces, and feathers.

The Litter mixture was slightly different between the Naturganic and BotaniGard experiments. The Naturganic experiments used Litter that had been directly collected from the hen houses. This was not an option when doing the BotaniGard experiments, instead wood shavings in a plastic container were placed where the hens could shed feathers and defecate onto them to create the same microbial environment as the previous substrate. Both the Litter mixtures were homogenized. The test treatments were applied directly to these substrates. In each trial, six glass petri dishes with an area of 63.62 cm^2 were used per substrate type, with three of the dishes serving as a treatment group, and three serving as a control group. Two trials per treatment were run, for a total of 12 dishes per treatment. There were a total of four trials run. In the case of the BotaniGard experiment one was run after spraying the bed bugs directly and the other was run after spraying the substrate and drying for 48 hours before adding the bed bugs and starting the experiment.

Mortality assessment

Mortality was recorded 2 hours, 1 day, 2 days, 3 days, 6 days, 9 days, 12 days, 15 days, and 20 days after treatment before the experiment was ended. Bed bug mortality was determined by gently nudging the bed bugs and observing movement. Bed bugs waving their antennae, shifting their stance, or moving were considered alive. They were considered dead when their legs had curled up and they no longer responded to stimulus. Exposure was terminated after 20 days. Any survivors were removed and frozen to later observe and compare to the bed bugs that died.

Statistical methods and calculations

Percent survival of control vs experimental groups for each treatment type was graphed and survival analyses were calculated using a Kaplan-Meier Estimation in JMP (include source information for the software) (Aak et al. 2017)). According to JMP statistical discovery Kaplan-Meier estimation is an approach to reliability analysis that estimates failure times and probabilities while not assuming an underlying probability distribution of failure times which makes it a nonparametric method. That means that it can be ideal for small-sample data like this. The log-rank test is used to compare the survival distributions of two or more unrelated groups, in this case the Naturganic, BotaniGard, and control groups. The statistical significance cut off was $P \leq 0.05$.

Imaging

Scanning electron microscopy (SEM) was used to take images of bed bug spiracles after Naturganic was used. Specimens were frozen at the end of the 20 day period regardless of if they had experienced mortality. Bed bug images were taken with a tabletop Hitachi TM4000PlusE II Scanning Electron Microscope at the University of California, Riverside Core Microscopy Facility.

Results

Naturganic

The control treatments for the Naturganic experiment experienced no mortality. When Naturganic was used on Litter there was also no mortality for any of the bed bugs in the treatment group (Kaplan–Meier log-rank test; Naturganic Litter vs. control: $\chi2= 0.00$, $P = 1.0$, DF = 1). This may be because the powder covered the bed bugs less effectively when dispersed and absorbed by the dirt, feces, straw, and feathers in the Litter. The Naturganic treatment group on Turf did decrease survival when compared to both the treatment group and the Litter group with some significance (Kaplan–Meier log-rank test; Naturganic Turf vs. control: $\chi2= 5.56$, $P = 0.0183$, DF = 1). When comparing Naturganic Litter to Naturganic Turf the results are much the same to when comparing Naturganic Turf and its control group, as Naturganic Litter had the same mortality rate of the control group (Kaplan–Meier log-rank test; Naturganic Litter vs. Naturganic Turf: $\chi 2$= 5.56, P = 0.0183 DF = 1). The P value for this experiment rejects the null hypothesis that Naturganic applied to Turf has no significant effect on the mortality of bed bugs.

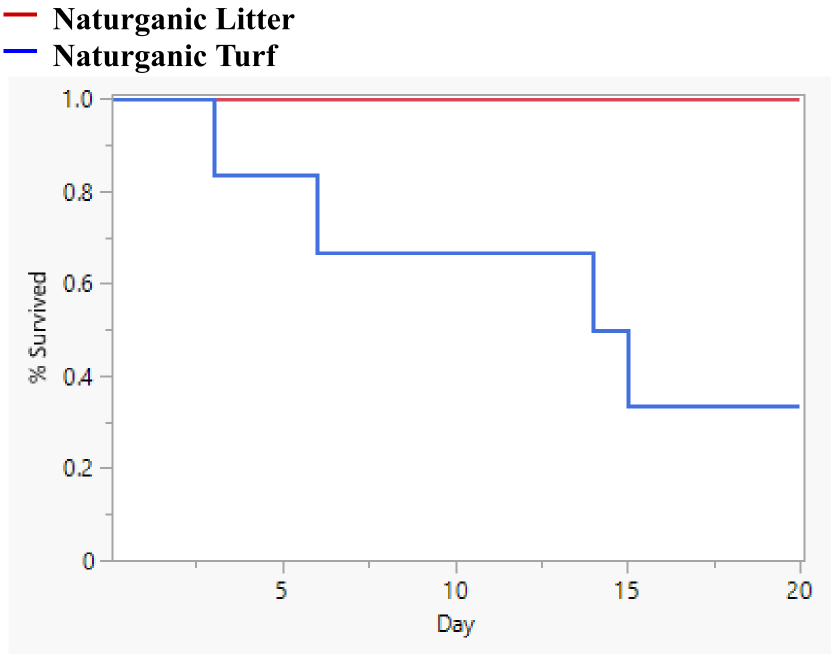

Figure 2: Naturganic percent survivability in Litter vs. Turf on a Day time scale

BotaniGard

The control treatments for the BotaniGard experiment experienced no mortality. The BotaniGard treatment group on Litter showed decreased survival of the bed bugs, though this effect was not significant (Kaplan–Meier log-rank test; BotaniGard Litter vs. control: $\chi 2$= 1.00, P = 0.3173, DF = 1). The chi-square value and the P value both indicate that this experiment fails to reject the null hypothesis, or that the pesticide on Litter has made no significant difference on the mortality of the bed bugs. The BotaniGard treatment group on Turf did decrease the survival of bed bugs as well, but again it was not significantly. (Kaplan–Meier log-rank test; BotaniGard Turf vs. control: $\chi 2$= 2.47, P = 0.1161, DF = 1). The chi-square value and the P value both indicate that this experiment fails to reject the null hypothesis, or that the pesticide on Turf has made no significant difference on the mortality of the bed bugs.

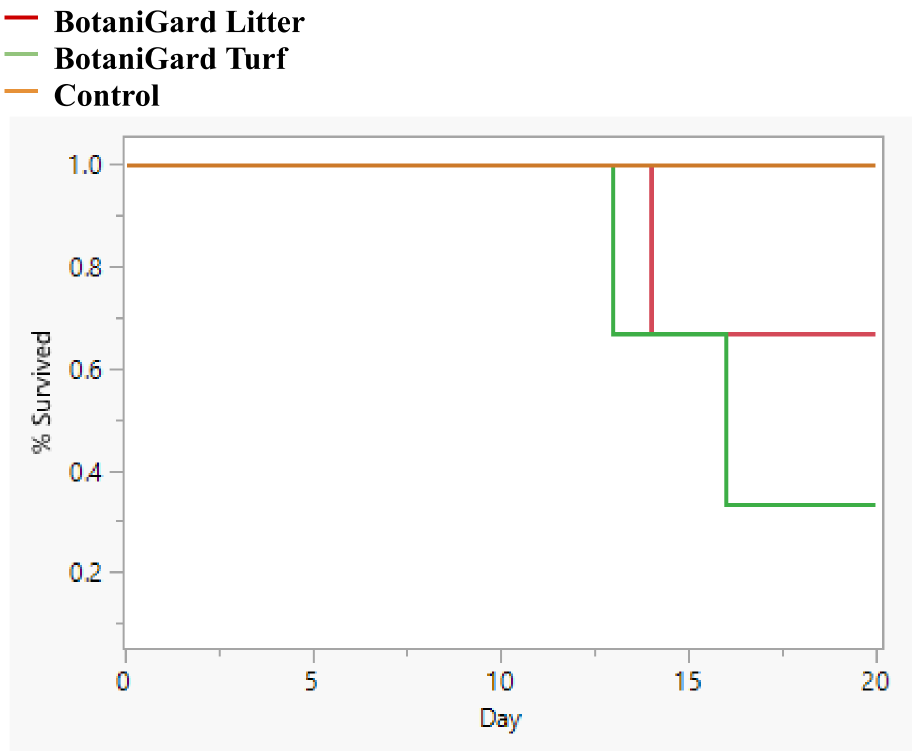

Figure 3: BotaniGard percent survivability in Litter vs. Turf on a Day time scale

Naturganic vs. BotaniGard

When comparing the four treatment groups to each other, there was no significant mortality rate (Naturganic Litter vs. Naturganic Turf vs. BotaniGard Litter vs. BotaniGard Turf: $\chi 2= 6.45$, $P = 0.0916$, $DF = 3$). We fail to reject the null hypothesis due to the P value being greater than 0.05.

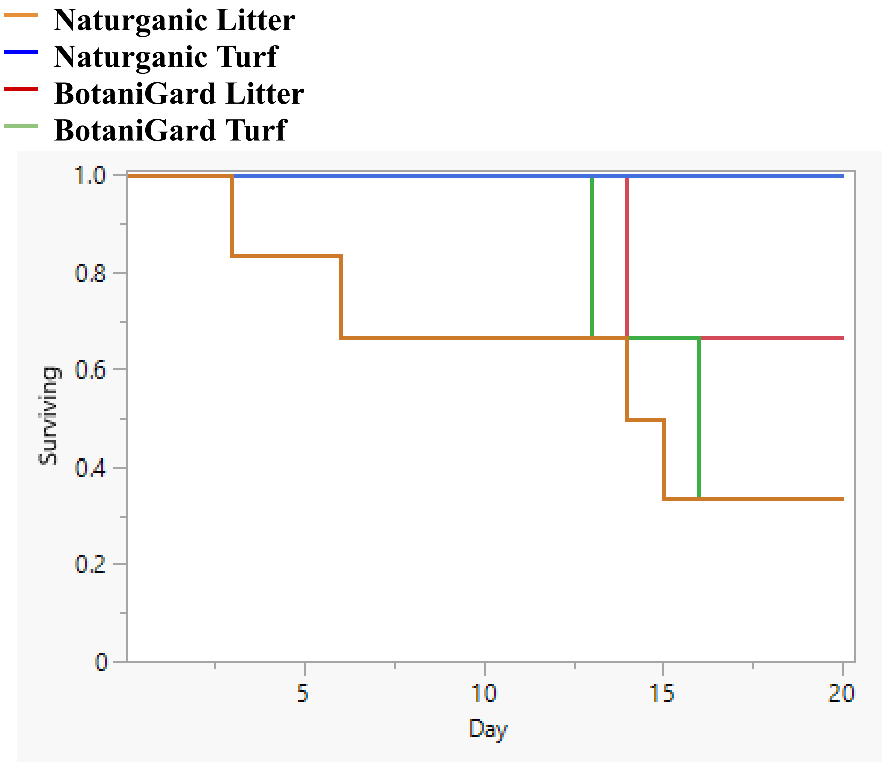

Figure 4: Naturganic and BotaniGard percent survivability in Litter vs. Turf on a Day time scale

Discussion

The hypothesis for this experiment was that the different environments provided would have different effects on the pesticides based on the amount of coverage they would provide, or how well they could host the conidia. Looking at the efficacy of the two products separately, only Naturganic on Turf had a statistically significant effect on the mortality of the bed bugs. It was also shown that the Turf environment used with Naturganic was significantly more effective than the Litter environment that showed no mortality. This could be due to the different amount of coverage that the Turf and the Litter provide, but more testing is needed. The mode of action of Naturganic is unknown, but it may work by clogging the spiracles on the bed bugs abdomens (which they breathe from), the Turf environment provided no shelter from the powder, so the bed bugs became significantly covered in it (Figure 5E, 5F), while the Litter environment provided a lot of shelter from the powder, which resulted in those bed bugs being relatively unscathed (Figure 5C, 5D). The difference in spiracle coverage, and its potential link to mortality call into question if the citronella is the key factor in killing the bed bugs in the first place. Naturganic is made up of 8% citronella oil, and 92% a mix of sodium bicarbonate, calcium silicate, and calcium carbonate. Seeing as the product is made up of a majority of these powders, they may be contributing significantly to bed bug mortality. Citronella oil may be acting as a general irritant to insects (that might successfully ward them off) (Telaumbanua et al., 2021), or even possibly a method to get the powder to stick better to the insects and clog their spiracles. To properly test the efficacy of Naturganic, additional tests on the product itself and in different environments are needed.

To test if the true cause of bed bug survival in the Litter group is the coverage amount, more bioassays would need to be conducted with different substrates. A plastic based substrate that would break up the powder and provide shelter would be ideal to determine if it was the amount of shelter provided or some other factor that determined the efficacy of the pest powder. To test Naturganic as a successful product, bioassays should be run in a standardized environment (preferrably using filter paper as the substrate, or if possible no substrate at all) where each powder is tested individually with and without the addition of citronella oil to determine if any one powder is the most effective, and if the citronella makes a significant difference. Since the formulation of the sodium bicarbonate, calcium silicate, and calcium carbonate mixture is proprietary knowledge, bioassays that explore different concentrations of each would also be interesting to test. It could also be valuable to test if Naturganic serves as a deterrent to bed bugs in the same way that citronella is often used as a deterrent against pest insects like mosquitoes. An experiment that tested if Naturganic discouraged bed bugs from seeking a blood meal would be very valuable because if it did prevent them from seeking a blood meal, then Naturganic could be used as a deterent that is also capable of killing insects. Even though Naturganic showed statistically significant results when it came to substrate types, that doesn't necessarily mean that it is an ideal, or effective pesticide. The limitations of this experiment fail to account for different circumstances, such as the reproduction rate of the bed bugs. This could result in an infestation growing out of control before the pesticide could be effective. There are many avenues open to testing this pesticide properly, but unfortunately they will not be pursued at this time due to time constraints of this project.

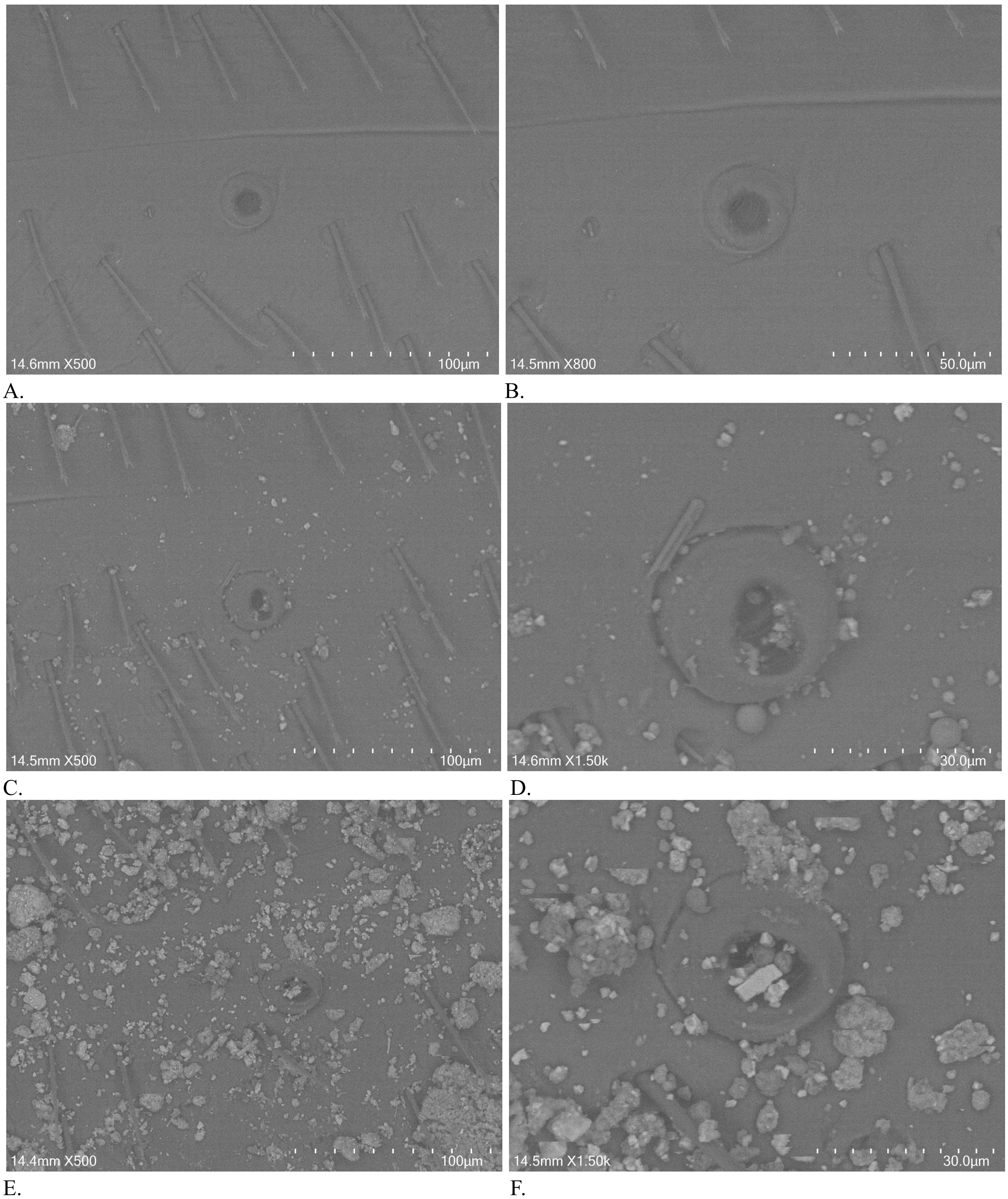

Figure 5. A. and B. Control group bed bug at 500x and 800x zoom respectively. C. and D. Naturganic bed bugs on Litter at 500x and 1500x zoom respectively. E. and F. Naturganic bed bugs on Turf at 500x and 1500x zoom respectively.

In contrast, BotaniGard was not effective in either environment. BotaniGard also did not have the same number of replicates as Naturganic due to difficulties in the first experiment run with BotaniGard. There was a change made after there was unexpectedly quick mortality in the treatment group that had been sprayed directly. There were two main issues, the first being that there was a very high mortality rate in the control group. The second issue was that in the first experiment using BotaniGard the spray was directly applied to the bed bugs, which is not common practice when it comes to using spray pesticides. All of the bed bugs died when sprayed, but it is more likely that they drowned in the solution since the conidia of *B. bassiana* is not what kills the bed bugs, but rather the fungus that forms from it. So, high mortality in the first few days before the fungus was even able to really start forming indicates an issue in the experimental design rather than the pesticide working as intended. Since it appeared that the bed bugs drowned in the pesticide, in the second replicate the BotaniGard was sprayed directly on the substrate and dried for 2 days before the bed bugs were introduced to the environment. This may have been too much time on the substrate before the bed bugs were introduced, and additional experiments are needed to optimize treatment timing. Humidity is another factor that impacts the efficacy of B. beauvaria, in previous experiments *B. bassiana* was kept above 40% relative humidity and was able to successfully work against pest insects (Aak et al., 2018; Acharya et al., 2015). Moist environments with high conidia levels were also successful in controlling pest populations (Mullens et al., 2012).

Overall these experiments had inconclusive results that would need to be expanded upon to draw any conclusions about the efficacy of the biopesticides on novel substrates for bed bug control. The pesticides may not be reliable as not all insects died before the experiment was concluded after 20 days. Running more replicates of the existing experiments for a longer amount of time would be a clear first step if there were no time constraints. In addition, increasing the amount of substrate types and bed bug life stages tested will provide more information about the role of environment on mode of action. A substrate that is high coverage with little to no microbial activity and a substrate that is low coverage with high microbial activity would be ideal to determine more specifically why some substrates would yield better results when using certain pesticides. Doing a separate experiment where the efficacy of BotaniGard was tested based on the drying time of the pesticide on the substrate would also help determine if the drying time makes a significant difference outside of the bed bugs drowning in the pesticide. With the time constraints involved in this project these experiments would need to be carried out in some future project.

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