Food Safe Alternatives to Methyl Bromide in Country Ham Production

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements of degree of

Master of Science
In
Animal and Poultry Sciences

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July 31, 2016
Blacksburg, VA

Keywords: ham mite, G.R.A.S, methyl bromide

Abstract

Dry cured meat production is a costly and long term investment for producers. Ham mites (*Tyrophagus putrescentiae)* are a common pest of dry cured products and cause devastating effects, potentially nullifying producers' investments due to loss of salable product, as well as regulatory concerns. Methyl bromide, a chemical fumigant used to control mite populations, is damaging to stratospheric ozone and will no longer be available. Presently, no alternative control measure has been approved to combat the ham mite; therefore, it is essential to identify potential alternatives. Interest in safe alternatives to control arthropod pest populations is gaining momentum, and garlic (*Allium sativum*) has been used to control other arthropod species including the northern fowl mite, mosquitos, and aphids. We chose to explore the efficacy of garlic juice in controlling *T. putrescentiae*. Using a choice test design, approximately 65% of the inoculated mites colonized on the control ham cubes, while no mites remained on garlic juice-dipped cubes. Garlic was ineffective when examined for volatile efficacy, but was effective in direct contact assays. However, as garlic juice was aged and diluted, efficacy was reduced even after treatments with antioxidants, metal chelators, and pH neutralization. In total, garlic juice acted as a short term repellent and showed efficacy in contact models, but application is time sensitive due to variable enzymatic degradation.

Dedication

This could not have happened without those who had faith in me and helped me reach my goals. However, this would not have happened without those who didn't, and attempted to impede my progress along the way. To both groups, thanks for the motivation.

Acknowledgements

God, family, and friends.

Many thanks to

Jesus Christ, my savior: You know more than anybody how close I was to losing my religion through this degree. I thank you for keeping me strong faithful throughout the process. I pray you continue building me into the man and servant that you desire. I thank you for the many blessings that you have bestowed upon Afton and myself. I promise we will do everything in our power to live our lives for you and only you.

Dr. Dave Gerrard, my advisor: I have had mentors that allowed me to just think inside the box and I have had many others that pushed me to think outside the box. However, you are the first to make me build my own box just so I could think both inside and outside of it, all the while simultaneously flipping it inside out. Although your style is not the easiest, it forced me to become a better scientist and definitely helped prepare me for the real world. My time working with you has made me realize that the most rewarding destinations are not always at the end of the easiest path. For that, I thank you.

Dr. Sally Johnson: for all your support, advice, and patience.

Dr. Jeff Bloomquist: for your insight on pest problems and entomological expertise.

Dr. Judy Mollet: for helping me get the entomology ball rolling.

Dr. Troy Anderson: for lending me a helping hand, knowledge, equipment, and your graduate students all in an effort to help control the ham mite. Without you and your lab, this would not have been possible.

Dr. Tom Phillips: for all mites, diet, and experimental design expertise.

Philene Vu: for your hands on support in trying to turn this meat head into an entomologist. I apologize for my ignorance and greatly appreciate your expertise in mite experimental design.

To all of my labmates: Sulaiman Matarneh, Eric England, Kristen Stufft, Bly Patterson, and Jordan Wicks. You all have become like family to me over the past two years. While we are probably the most diverse and dysfunctional family I have ever known, somehow we have managed to function together through it all.

Skylar Workman, my undergraduate volunteer: while our time together was short, your willingness to help was pinnacle in completion of this project.

Patricia Williams: words cannot express how much I appreciate everything over the years.

Afton Olivia Preisser, my wife: it takes a very special kind of woman to put up with somebody like me. I never would have thought that I would find one willing yet eager to deal with my ways. I could not have gotten through this without you and your daily support. God was definitely looking out for me when he sat us both down in the same intro class.

My family: for all of the love and support during this experience. **Mom and Paul:** Even though you two may not have always understood or agreed with me becoming a "professional student", you were always there for me. I would not have made it here without either of you.

Virginia Pesticide Council: without your financial backing, this wouldn't have happened.

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Chapter 1. Introduction and Literature Review of Country Ham Industry

Introduction

Tyrophagus putrescentiae, more commonly known as the ham or cheese mite, is a common pest of dry cured meat products. These parasites cause food safety concerns in the form of allergens and are difficult to control due to the vulnerability of country ham during various stages of production. For years, methyl bromide has been used as an effective fumigant for controlling ham mites on dry-cured hams. Recently, the industry has faced the loss of this valuable chemical. Due to damaging effects on the stratospheric ozone layer, methyl bromide is being eliminated in accordance to the Montreal Protocol's Clean Air Act. Therefore, effective methyl bromide replacements are necessary so producers can continue to sell safe and wholesome country ham products in the foreseeable future. Current alternatives include: other fumigants, as well as food-safe coatings and compounds.

Regulatory Development

Methyl bromide has been the gold-standard fumigant in the ham industry for well over 40 years (Sekhon et al., 2010). Applied as a fumigant, methyl bromide is used against multiple pests ranging from fungi to rodents; thus, is considered a broad spectrum pesticide (Sekhon et al., 2010). It has also been implemented in a variety of commodities ranging from vegetables to arboriculture (Noling & Becker, 1994). Regardless of methyl bromide effectiveness and its versatility, it is one of the leading contributors to ozone depletion, making safe alternatives essential (Marriott & Schilling, 2004).

The Clean Air Act of the Montreal Protocol established new regulations governing the future of methyl bromide, which is now considered a Class I ozone depleting substance (EPA, 2007). At present, the cost of methyl bromide has reached a point where it is no longer readily available. Since 1999, the purchase price of methyl bromide has increased from $2.50/lb. to $20/lb. (Osteen, 2003). It is unknown when methyl bromide usage will be banned completely, but it is currently the only chemical available for effective control of ham mites (EPA, 2010). Therefore, there is some urgency in identifying alternatives to methyl bromide.

Country Ham Industry Overview

History

As with many historical facts, there are discrepancies in the origins of the salting process as a food preservation practice. Mesopotamians are thought to be the first culture to use salting as a form of preservation, which was likely discovered by necessity (Nummer & Andress, 2002). Salt curing is a process used on a number of food items, including but not restricted to fish, red meats and vegetables as early as 3000 B.C.E. (Pariza, 1997). Cato, a Roman author, also documented the importance of using salt to preserve food around 234-149 B.C.E. (Nummer & Andress, 2002). Regardless of the inventor, the process of salting food has been around for a longtime as a means to preserve food against deterioration and spoilage.

Current Demographics

Salt curing of meats has evolved from being an essential means of preservation to a relatively small and diverse niche industry that produces a wide variety of products with highly variable final characteristics. In fact, salt cured meats are mainly considered a delicacy. The majority of the hams produced across the globe originate from the “ham belt”, which includes

portions of Europe, China, and North America mainly because these countries provide the best climate and conditions for salt cured ham production (Tobergte & Curtis, 2013).

The process of salt curing is often generalized to hams, but other products are cured using salt, including fish, duck breast, spalla (pork shoulder), pancetta (pork belly), lardo (pork fat), capocollo (pork neck shoulder junction), sopresatta and other sausages. In Asia, there are two major types of ham, Jinhua and Yunnan hams (Tobergte & Curtis, 2013). Common Spanish and European hams include: the Westphalian (Germany), Black Forest (Germany), Prosciutto/Parma (Italy), Culatello, Jamon Iberico (Spain), and Jamon Serrano (Spain) (Tobergte & Curtis, 2013).

North America-specific, dry-cured hams (country hams) are most often produced in Virginia, North Carolina, West Virginia, Kentucky, Tennessee, Georgia, and Missouri (Rentfrow et al., 2008). However, there are two main styles produced, the South East (SE) Virginia-style or the South West (SW) Virginia-style (Graham et al., 2012). Originating around Smithfield, Virginia, the SE style is most commonly fabricated and consumed, indicative of the Smithfield ham. The main difference between the two hams is the SE hams includes a smoke flavor and the SW version generally does not. The SW ham usually involves applying a sucrose-based coating instead of smoking. This often consists of a sweet liquid mixed with a spice, most likely some type of honey or molasses base. While both have their advantages and disadvantages, it is important to understand the differences in initial processing, as it plays a key role in how and why ham mite problems can occur.

Production Process

Ham production practices create an ideal environment for ham mites and other pests. Indeed, certain aspects of production are essential for quality ham production. Even so, however, particular segments of the process promote ham mite survival and growth. First, the process is

very long, at least relative to other processed meats procedures, sometimes spanning over multiple years. During that time, there are many variables that can be potentially deleterious to the process. Common examples include losses associated with pests, inadequate cure uptake, ham sour, and other unforeseen issues related to storage time. This additional time and overhead is realized in the cost of the product. Some specialized hams such as Iberico Prosciutto for example, can reach $1500 U.S. dollars per ham or about $150/lb. Although typical country hams are less valuable, the potential losses to producers are nonetheless severe.

All meat can be cured. For brevity, hams will be used as an example and general term. First, fresh raw products must be sourced (Grahm et al., 2012). Traditionally, hams for dry-curing weigh from 6.8 to 11.3 kilograms. Salting is key to ensuring a safe and wholesome product. Hams need to lose a minimum of 18% of their green or fresh weight and contain 4% salt in the final product (Rentfrow et al., 2012; Tobergte & Curtis, 2013). The salt mixture varies with producer and consists of a mixture of salt, sugar, spices, and cure. The general recommendation is an 8-2-2 mixture, consisting of 8 lbs (3.6 kg) salt, 2 lbs (0.9 kg) sugar, and 2 oz (56.7 g) cure for every 100 pounds of green product, respectively (Grahm et al., 2012). Country style hams are generally salted for about 1.5 - 2 days per pound green weight in a cooled environment (Voltz and Harvell, 1999; USDA, 1999).

The next step is the equalization process. At this point, hams are equilibrated to about -12.2 to -6.6 ºC warmer environment so salt can evenly distribute throughout (Tobergte & Curtis, 2013). It is critical the salt reaches all portions of the ham equally or the entire ham will be subject to spoilage.

After equalization, hams are generally cold smoked to ensure the internal temp resides below 32.22º C (Grahm et al., 2012). If the temperature isn't properly controlled, microbial

growth occurs and product quality impacted. It is also important to note that this process is not a cook step, and 'smoked' doesn't imply it is ready to eat, or RTE. Many people don't realize that most hams require cooking prior to consumption, even though they are often shelf-stable.

The final step before sale is the curing or "summer sweat" step (Tobergte & Curtis, 2013). It is during this step that the ham gets its unique flavor and quality. Hams are stored at temperatures that simulate a warm summer day, which historically is considered optimal curing conditions. Smaller facilities generally use ambient air temperatures, but larger operations produce hams year around and have climate controlled rooms allowing for relative humidity and temperature regulation. It is ideal to have room around 65% relative humidity, as it is important to final product quality (Rentfrow et al., 2012). The cure process can range from a few months, to a few years depending on the product quality specifications. The extended process and relative humidity utilized in country ham production, especially the summer sweat, provide an ideal environment for ham mite infestations. Ham spacing, wooden equipment and rooms exacerbate the problem.

Arthropod Pests

One of biggest issues in country ham production is the presence of pests in or on the product. Hams houses provide the environment and necessary food source to supply arthropod infestations that can continue undetected for long periods of time. Unfortunately, the age and style of many traditional ham houses lends additional opportunity to pest problems. When these factors are combined with high traffic and annual use of most ham houses, pest problems arise.

Other Stored Meat Pests

The larder beetle (*Dermestes lardarius* L.), red legged ham beetle (*Necrobia rufipes*), and skipper (*Piophila casei*) are common pests of dry cured meat products (Grahm et al., 2012).

Some cause considerable damage, but in ways unlike the ham mite. Moreover, control methods differ greatly from those used against ham mites. Regardless, it is important to effectively differentiate the following pest from the ham mite.

Cheese Skipper-

The skipper is an insect most prone to be a problem with hams stored outside, but can exist in the most modern ham houses. The skipper gets its name from the hopping action of the larvae (Grahm et al., 2012). The larvae tunnel through hams, which makes the interior vulnerable to other insects or spoilage bacteria. Skipper infestation is easily observed by a gooey-like texture on the surface of hams. The skipper has two wings and are 1/3 the size of a typical house fly and multiply very rapidly. Hams with skippers should be heavily trimmed around tunnels or discarded (Grahm et al., 2012).

Larder Beetle-

Larder beetles are yellow/brown in color and are usually 8.5 mm (Grahm et al., 2012). The larvae feed underneath the surface of the ham which prevents ham spoilage, but spots where larder beetles are found should be removed.

Red-Legged Ham Beetle-

The Red-legged ham beetle larvae are a dark red and about 8.46 mm long. Adult beetles feed on the ham surface and are bluish green in color and measure about 6.35 mm long (Simmons & Ellington, 1925). Like the skipper, they tunnel through the meat; however, their tunnels serve only to dry the meat and damage resulting from a beetle infestation are simply trimmed and properly cooked before consumption (Grahm et al., 2012).

All of the aforementioned arthropods are serious pests of country ham and the damage caused by each is different than the ham mite.

Ham Mite

Mite Impact on the Industry

Ham mites are a significant problem to the industry, not because they degrade the production, but because they are considered a zero tolerance due to the allergenic nature of the mite. Mite presence causes surface damage and can periodically shut down an operation due to current regulatory tolerances (USDA, 1999). Interest in alternative strategies to control these acari has been proposed by integrated management systems that include country ham IPM and methyl bromide replacement options (Zhao et al., 2016).

Commonly referred to as “barn allergy”, storage mites produce numerous allergens that can affect a wide range of people and pet species (Cuthbert et al., 1979). Acari species including *Tyrophagus*, commonly cause dermatitis and respiratory issues through the allergens they shed in agriculture industries (Wraith et al., 1979). Under certain cases, mite allergens can cause systemic anaphylaxis, making their presence highly regulated (Matsumoto et al., 1996).

A study was conducted on thirty-four U.S. ham producers to determine the prevalence of ham mites (Rentfrow et al., 2008). Of those producers surveyed, twenty-five plants had problems with ham mites and fifteen of which had problems after 6-months of curing. Curiously, ten of the producers had mite problems before the product had cured 6-months. This information shows the prevalence of mites in the industry, and suggests mites are both a long- and short-term problem.

Mite Biology

Ham mite (*Tyrophagus putrescentiae*) are common pests in stored meats, cheeses, flour, grains, and animal feed (Rodriguez & Rodriguez, 1987; Chambers, 2002). Because of their size, ham mites are often mistaken for dust mites, but they are actually mold mites (Mueller et al., 1997), and can readily infest any product with adequate fat, protein, or moisture content (Mueller et al., 1997).

Belonging to the acaridae family, mites are near microscopic (1/2 mm) in size and white colored and tend to reflect the color of food they consume (Zhao et al., 2016). Ham mites generally appear as off-white particles on the ham surface during the curing process. They are more noticeable as the colony becomes established because of the residual exoskeletons and newly formed, developing mites. A distinct odor is associated with mite infestations, sometimes described as sweet or minty odor and experienced producers can smell infestations prior to being visible to the naked eye (Townsend, 2008).

Under optimal conditions mites live about 31 days, and as temperatures decrease, the mite life cycle reacts inversely (Sass, 2006; Mueller et al., 1997). Inactive mites can withstand temperatures down to 0 ºC, but do not development below 10 ºC (Mueller et al., 1997). Each female has the potential to lay approximately 800 eggs in her lifetime under ideal conditions (Townsend, 2008). Mites prefer a warm moist environment and tend to be more productive on less desiccated product surfaces. The ham mite life-cycle consists of the egg, larva, nymph, and adult (Townsend, 2008).

Dry cured products are formed under conditions of around 75˚F (23.8˚C) and 65% humidity depending on the production system (Rentfrow et al., 2012). When the ham begins the essential cure or summer sweat process, moisture is released from lean surfaces providing a

moist and nutrient rich food source for the ham mite. This moisture loss periodically softens the surface and causes mold growth and allows mites to flourish.

Pest Management

Integrated Pest Management

Because dry cured ham production and optimal mite growth share similar optimum conditions, a great deal of work has been put into altering or improving common production practices in an effort to reduce mite occurrence. Integrated pest management (IPM) is a systematic or stepwise method for controlling pests that involves the use of multiple practices that help reduce a pest population. IPM is strategic accumulation of methods that is utilized in many pest management protocols today. IPM practices are configured differently for all pests, yet have the common goal of controlling pest populations.

Rentfrow et al., (2015) provided a comprehensive review of management practices that help prevent the occurrence of ham mites. Prevention for ham mites begin with the exterior of the operation. Producers should seal off access areas or inhabitable spaces that mites may use, minimize plant traffic, and enforce biosecurity measures at all times. For non-inspected operations, suitable insecticides may be used to treat empty rooms (Rentfrow et al., 2015). Alteration of humidity and temperature help control mite development (Barker, 1966). Proper sanitation begins with removing debris, weeds, and product trimmings from the ham house because it eliminates inhabitable vectors (Rentfrow et al., 2015). Monitoring mite populations is conducted using visual and olfactory inspections of the ham the storage area. Traps are also available and can be utilized for early mite detection.

These procedures can aid in pest reduction when combined with other measures such as chemical control. Alternative fumigants such as phosphine and sulfuryl fluoride **(SF)** have been used to further control pest populations. However, neither are ideal because both are corrosive and deleterious to the facility and fail to control egg stages (sulfuryl fluoride).

Alternative Fumigant Control

Phosphine

Phosphine fumigation has been studied as a potential substitute for methyl bromide in the country ham industry because of its successes in other stored commodities (Sekhon et al., 2010). Phosphine is easily implemented, relatively inexpensive, and delivers a strong knockdown (Zuryn et al., 2008). However, phosphine fumigation has negative side effects including: pest resistance, flammability, and corrosive to metals in ham houses (Price and Mills, 1988; Zettler, et al., 1989; Brigham, 1998).

Phosphine is extremely potent for pests utilizing oxidative respiration, but less so to those capable of functioning in low oxygen conditions (Sekhon et al., 2010). This occurs because phosphine alters oxygen metabolism, forming deleterious oxyradicals, which in turn alters required enzymatic function leading to metabolic disorders (Zuryn et al., 2008). Because of these qualities, phosphine is effective in controlling multiple insect growth stages (Bell, 1976)

No dedicated legal limit is set for residual phosphine, but based on other industries it is probably very close to 0.01 ppm (Sekhon et al., 2010). Based on this limit, phosphine can be fumigated at concentrations up to 1000 ppm and still be below the legal limit for safe consumption with minimal sensory aromatic quality impacts (Sekhon et al., 2010). At these

levels, residual levels are below the 0.01 ppm limit and effective on all mite life stages (Schilling et al., 2010).

Sulfuryl fluoride

Sulfuryl fluoride is currently under investigation for ham mite control (Sekhon et al., 2010). The same study tested various fumigation concentrations and volatile flavor concentrations of sulfuryl fluoride and fluoride ranging from (0, 12, 24, 36 and 72 mg/L). Results show that both fluoride and SF concentrations became greater as fumigant concentration increases, but the legal limits of 20 and 0.01 ppm were never crossed. Aromatic differences were minimal, but product oxidation compounds were increased in the highest fumigation concentration. In total, there were minimal aroma/flavor differences in hams fumigated with the four lowest concentrations, and no safety concerns for consumption were noted. Further, SF is unable to control egg stages even after multiple applications. This makes the use of SF a poor substitute for methyl bromide (Sekhon et al., 2010; Zhao et al., 2016).

Due to problems associated with chemical alternatives and consumer trends, interest in safer control measures is expanding. Currently, alternatives include biological control, physical modification, ozone fumigation, CO_2 fumigation, food grade coatings, and botanical insecticides.

Alternative Control

Biological Control

Biological control measures are a useful tool against pest problems, especially in IPM. (*Cheyletus eruditus)* is a common mite predator that has been utilized in stored grain applications (Zdárková, 1998); however, control abilities vary with environmental conditions and relative

prey proportions (Lemus, 2014; Zdárková, 1998). Unfortunately, due to differences in production practices and biological requirements, *C. eruditus* was less effective in dry cured ham production than in the grain industry (Escudero & López, 2001).

Physical Methods

Temperature reduction and irradiation have been proposed to combat the ham mite as they have been applied in many other IPM protocols (Zhao et al., 2016). Ham mite egg mortality occurs at temperatures of -15º C and -5º C after 24 h and 24 d time (Boczek, 1991). However, Arnau & Guerrero, (1994) were unable to produce egg mortality after 48 h at -25º C. Unfortunately, extreme temperature reduction can be deleterious to ham quality and attempts to use high heat treatments have led to safety concerns (Zhao et al., 2016). In total, conflicting results, safety concerns, and quality impediments make temperature modification impractical in the control of ham mites. Other practices such as ultraviolet-c irradiation have been attempted and proven effective on ham mites without quality deterioration. However, the cost associated with necessary equipment and implementation is prohibitive for most producers (Zhao et al., 2016).

Ozone Modification

In *in vitro* studies, application of 175 ppm ozone concentration for 48 h produced 100% mortality of ham mite at all life stages (Sekhon et al., 2010). Unfortunately, the penetrative properties of ozone are minimal, meaning it may not be effective on pests residing in fissures below the ham surface. Additionally, ozone increased ham oxidation, but not to consumer detectable levels. Further research in full scale operations is necessary before considered a viable

alternative. Ozone is highly corrosive to equipment and may have some difficulties in being adapted, if proven effective.

CO_2 Fumigation

Schilling et al. (2010) examined the efficacy of atmospheric modification through CO_2 gas, a common ingredient in modified atmosphere packaging. After a 144 hr exposure, at 60% CO_2, complete mortality occurred, regardless of life stages. Even so, however, a 144 hr fumigation protocol is industrially unfeasible and impractical given this would greatly reduce plant productivity. Furthermore, safety concerns arise as high concentration CO_2 fumigation can be dangerous even to surrounding organisms.

Food Grade Coatings

In the Spanish dry cured ham industry, it is a common practice to coat products with a lard-based paste to help protect hams against a number of storage-related issues (García, 2004). Food grade coatings are also utilized in other industries such as fruits, vegetables, and meat products (Zhao et al., 2016). Recently, Zhao et al., (2016) showed that mite growth was inhibited through application of food grade coatings including: lard, mineral oil, glycerin, propylene glycol and potassium sorbate. Ham cubes were dipped directly into mineral oil, propylene glycol, 10% potassium sorbate solution, and glycerin each for 1 min and allowed to drip on a mesh colander for 1 min. Lard was applied directly by rubbing a thin layer to cover the entire area. Trials lasted 21 days to allow for mite reproduction and population data. Results indicated that combinations of propylene glycol, xanthan gum and carrageenan + propylene glycol alginate work well as a preventative against ham mite colonization in a lab setting and allowed moisture

and oxygen exchange. Additional testing will be necessary to see if this approach can be scaled to large production facilities.

Monoterpenes

Acaricidal effectiveness of some plant extracts and essential oils are thought to result from monoterpenoids, a form of terpene comprised of two isoprene units (Gulati & Mathur, 1995). These compounds are often associated with fruits and plant essential oils, and Castan (2001) found that seven naturally occurring monoterpenes (pulegone, eucalyptol, linalool, fenchone, menthone, a-terpinene and gterpinene) had a high acaricidal effectiveness from vapor action with LC_{90} values of 14 ml/l. Egg hatching data was not recorded, but larvae and males yielded a higher mortality rate than females. This phenomenon could be attributed to surface/volume ratio differences between males and females, allowing faster moisture loss. However, death by respiratory interference could not be ruled out.

G.R.A.S Acaricides

Along with food grade coatings, alternative compounds of interest include generally recognized as safe (G.R.A.S), natural, or botanical compounds. Many common insecticides and repellents are derived from plant sources, an example being pyrethrins from Chrysanthemum flowers. As with many industries today, consumers and producers share a common interest in utilizing cleaner and more environmentally friendly compounds to help control pest problems. Accordingly, multiple GRAS compounds have been tested and shown promise against the ham mite. However, implementation without altering ham quality is challenging for such compounds, as many produce strong flavors and volatile characteristics that could negatively affect quality.

Pine Essential Oil

Macchioni et al. (2002) explored the efficacy of pine essential oils extracted from a variety of pine species on (*T. putrescentiae)* on mite survival. In order to understand pine oil efficacy, the main constituents were examined. Oils derived from *P. pinea*, *P. halepensis*, and *P. pinaster* provided respectable arcicidal properties. Components of *P. pinea* branches that were tested included R-pinene, â-caryophyllene, myrcene, 1,8-cineole, and limonene. Oils were examined via aerial diffusion. All tested oils exhibited effectiveness, but *P. pinea* oil and its two components 1,8-cineole and limonene proved to be the most effective. All three offered 100% acaricidal action.

Fennel Seed Oil

In Asia, fennel *(Foeniculum vulgare)* is used for its medicinal properties due to trans-anethole, estragole, d-limonene, (+)-fenchone, α-pinene, γ-terpinene and *p*-cymene components (Kim et al., 2004). Some of these components have insecticidal attributes (Kim & Ahn, 2001). Lee et al. (2006) examined the effectiveness of fennel oils and constituents on ham mites *(T. putrescentiae)*. Multiple compounds proved to be effective based on LD_{50} values in direct contact studies after spectroscopic analyses. Relative toxicity when compared to benzyl benzoate, dibutyl phthalate and *N*,*N*-diethyl-*m*-toluamide (DEET) controls, were as follows: carvone [dihydrocarvone , (+)-carvone , (?)-carvone] and naphthalene. The study concluded that fennel and constituents warrant further studies to better test their potential as acaricidal options.

Cinnamaldehyde

Kim et al. (2004) tested the efficacy of cinnamaldehyde (an organic compound associated with cinnamon flavor and odor) and its constituents on ham mites *(T. putrescentiae)*,

using benzyl benzoate, DEET, and dibutyl phthalate as controls. Direct contact and volatile studies were based on LD_{50} values after 24 hrs. A variety of compounds proved to be more toxic than controls, including: cinnamyl acetate, cinnamaldehyde, benzaldehyde, 3-phenylpropionaldehyde, cinnamyl alcohol, salicylaldehyde, and (*E*)-2-hydroxycinnamic acid. The (*E*)-cinnamaldehyde, cinnamyl alcohol and salicylaldehyde appeared to be most effective in sealed containers, attributing a majority of the efficacy to volatiles. In conclusion, cinnammaldehyde constituents warrant further investigation as mite treatments.

Thyme

Jeong et al. (2008) tested thyme *(Tymus vulgaris)* oil and some of its components against the ham mite *(Tyrophagus putrescentiae)*. Using an impregnated fabric disk bioassay, they determined that responses varied based on dosage and chemical components. The 50% lethal dose (LC_{50}) value of the T. vulgaris oil against T. putrescentiae was 10.2 g/cm^2. Carvacrol was the most potent component, indicating carvacrol is the leading contributor to thyme toxicity even though only comprising 14.2% of the compound.

Peppermint Oil

The main component of *M. piperita* oil is menthol which is antimicrobial, larvicidal, and has repellent properties (Ansari et al., 2000; Osawa et al., 1998). Utilizing fumigant and contact bioassays, Park et al. (2014) examined the acaricidal activities of peppermint oil and menthol isomers against the ham mite *(T. putrescentiae)* using benzyl benzoate as controls. Results showed that menthol is 15 times more active than benzyl benzoate, suggesting there could be potential for future testing of peppermint oil and its menthol isomers.

Either through volatile or contact exposure, all of the aforementioned botanicals affect mite mortality to some extent in advanced stage. However, the common inadequacy shown by all botanical compounds is low egg mortality. Without egg functionality, a substance is not typically deemed suitable as a control measure. Therefore, further investigation into botanical alternatives is necessary to find viable methyl bromide replacements. Curiously, garlic has been utilized in other pest and commodity programs, but has not been examined for ham mite control.

Garlic Insecticidal Properties

Garlic is part of the allium family, which includes onions, leeks and shallots. Allium products have culinary popularity due to their pungent flavor and aromas caused by organosulfur compounds, which tend to be especially high in the allium family (Meiser et al., 2014; Wang et al., 1998). Although unstable, garlic contains multiple insecticidal intermediates consisting of allicin (Singh & Singh, 1996) and diallyl disulphide and diallyl trisulphide (Bhatnagar-Thomas & Pal, 1974b).

The insecticidal properties of garlic *(Allium sativum)* have been used against multiple insect pests. The relationship between garlic's distinctive odor that is similar to that of Chara L., an alga under investigation as biological mosquito larvicide, sparked the interest in using garlic to fight mosquito populations (Amonkar & Reeves, 1970). Nzanza & Mashela, (2012) showed the efficacy of wild garlic as a bio-pesticide against whiteflies and aphids. In Europe, the desire to improve sustainable agriculture practices has become a widespread goal and one method of improving sustainability is through the use of botanical extracts such as garlic.

Birrenkott et al. (2000) utilized garlic oil to reduce northern fowl mite populations in laying hens. The parasitic northern fowl mite causes a reduction in laying hen productivity by

causing anemia and even death, so 10% garlic oil solution was introduced through topical application. The rationale behind selecting garlic as a control method was the desire to find safer alternatives to the carbaryl (Sevin) insecticide typically used (Crystal & Demilo, 1988; Fletcher & Axtell, 1991). This is because carbaryl application warrants special care around eggs, feed, and water (Ivey et al. 1984). Birrenkott et al. (2000) found a significant reduction in mite populations in the garlic-treated birds compared to controls. In addition, George et al., (2010) utilized garlic against the poultry red mite *(Dermanyssus gallinae),* which also causes irritation and anemic problems in laying hens. Results showed that garlic was about 90% effective against adults and 70% effective on younger stages.

According to Prowse et al. (2006), components of garlic function as enzyme inhibitors, and one of the main enzymes inhibited being acetylcholinesterase. This inhibition is attributed to allicin, diallyl disulphide and diallyl trisulphide (Singh & Singh, 1996; Bhatnagar-Thomas & Pal, 1974b). Polysulphide groups composing these compounds may be responsible for the reactive actions and eventual toxicity (Prowse et al., 2006). Polysulphide groups cross-link with thiol constituents comprising enzyme frameworks, leading to changes in protein shape, function, and eventually denaturation and inactivation (Halliwell & Gutteridge, 1999).

One of the main complications when using garlic is the inconsistency of components in the end product and the efficacy of garlic derivatives differ due to variable enzymatic breakdown (Prowse et al., 2006). To confound that, different orders, species, and sub-species life stages react differently to the same compound. This variation impedes the successful production and distribution of garlic based compounds as viable insecticidal products in many cases.

Garlic juice is proven effective in controlling other mite and insect species. It is a common food ingredient and may have less impact on country ham flavor than aforementioned essential oils. However, further testing would be necessary to ensure quality impacts are minimal and see if natural variation plays a role in ham mite efficacy.

Conclusion

Country ham production is a high risk, high reward business. With the new Clean Air Act threatening to eliminate the use of methyl bromide for control of ham mites, the risk for ham producers has increased profoundly. Therefore, effective food-safe alternatives to methyl bromide need to be explored for efficacy against the ham mite and garlic may be a good candidate for exploration.

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Chapter 2. Acaricidal effects of fresh garlic juice on adult *Tyrophagus putrescentiae*

Abstract

Dry cured meat production is a costly and long term investment for producers. Ham mites (*Tyrophagus putrescentiae)* are a common pest of dry cured products and cause devastating effects, potentially nullifying producers' investments due to regulatory concerns. Methyl bromide, a chemical fumigant used to control mite populations, is damaging to stratospheric ozone and will no longer be available. Presently, no alternative control measure has been approved to combat the ham mite, Therefore, it is essential to identify potential alternatives. Interest in safe alternatives to control arthropod pest populations is gaining momentum. Garlic (*Allium sativum*) is used to control other arthropod species including the northern fowl mite, mosquitos, and aphids. We chose to explore the efficacy of garlic juice in controlling *T. putrescentiae*. Using a choice test experimental design, approximately 65% of the inoculated mites colonized on the control ham cubes, while no mites remained on garlic juice-dipped cubes. Garlic was ineffective when examined for volatile efficacy, but was highly functional in direct contact assays. However, as garlic juice was aged and diluted, efficacy was reduced even after treatments of antioxidants, metal chelators, and pH neutralization in efforts to extend functionality. In total, garlic juice acted as a short term repellent and showed efficacy in contact models, but application is time sensitive due to variable enzymatic degradation.

Introduction

The ham mite *(Tyrophagus putrescentiae)* is a common pest of country cured hams. Control of mites has long resided in the use of methyl bromide (MB) as a fumigant. However, damaging environmental effects to the ozone have since rendered MB obsolete under the Montreal Protocol's Clean Air Act. Therefore, effective alternative to methyl bromide are necessary to ensure producers can continue distributing safe and wholesome country ham products into the foreseeable future. Current alternative areas of interest include: fumigants, food-safe coatings, and natural products and related compounds.

Previous studies have shown the efficacy of naturally-derived substances such as pine, cinnamon, peppermint, and thyme (Macchioni et al., 2002; H. S. Lee, 2008; Kim et al., 2004; Park et al., 2014). Based on the aforementioned studies and information acquired from other pest species problems (Birrenkott et al., 2000), garlic was chosen to test against the ham mite. The central hypothesis is that ham mites can be controlled by incorporating generally recognized as safe (GRAS) compounds, such as garlic juice on the surface of hams. The overall objective of this application is to keep the dry cured ham industry sustainable into the foreseeable future by exploring alternative strategies to mitigate mite infestations in dry cured ham processing. Simply put, our methods are comparing the acaricidal effectiveness of GRAS compounds within a controlled and industry-relavant environment. Current focus is on freshly extracted garlic juice with efficacy indicators of living (mobile), dead (non-mobile and no functioning limbs), or immobilized (non-mobile but limbs functioning).

Materials and Methods

Mite Colonization

Tyrophagus putrescentiae specimens were obtained from Dr. Thomas Phillips at Kansas State University, Manhattan, Kansas, USA and raised in 946.4 ml glass jars sealed with filter paper to allow for gas exchange. Colonies were fed specialized diet bi-weekly and housed in a growth chamber held at approximately 25º C (+/- 5º C) and 50% (+/- 10 %) humidity. Holding chambers were housed in a separately ventilated room from treatment chambers to prevent exposure to potential volatiles.

Mite diet was also provided by Dr. Phillips. While ingredients remained consistent with the original recipe, quantities were altered to better suit Virginia Tech laboratory conditions. Ingredients included 475 ml distilled water, Beneful® dog food, 25 ml glycerol (99.9%), brewer's yeast, agar, alphacel (non-nutritive bulk cellulose), insect vitamin mix, 15% methyl-*p*-hydroxybenzoate in 95% ethanol. The effective diet duration is highly dependent on environmental temperature and relative humidity. When the room was held at 25 ºC and 60% relative humidity, mite diet was made approximately every 1.5 weeks on average. When humidity was increased, effective diet duration was decreased due to increased consumption of the mites.

Mite diet was comprised of 320 g Beneful (Purina Animal Nutrition LLC, Minneapolis MN), original dog food, 1000 ml distilled water, 63 g (99.9%) glycerol, 10 g brewer's yeast, 10 g agar, 10 g alphacel (non-nutritive bulk cellulose), 10 g insect vitamin mix, 7.9 ml of 15% methyl-P-hydroxybenzoate in 95% ethanol. Water and dog food were placed into a 3.8-liter pot. The pot was heated to render down dog food. Jars were filled with dry dog food approximately half way. Once the wet portion is simmering, inclusion of remaining ingredients is completed. thoroughly mixing between each. Once mixed, liquid portion is distributed between jars allocating as needed for growth. The greater the wet to dry ratio, the faster mites repopulate. After distribution, mix wet and dry portion thoroughly forming a cone shape with the narrow

side on the bottom and wider portion at the top of the jar. Let cool for approximately 1 hr and inoculate with three tablespoons of mites from previous feeding.

Ham Cube Samples

Salt cured ham used for experimental assays were made in the Virginia Tech Meat Center. Hams were made using an 8-2-2 recipe, containing 8 pounds (3.6 kg) salt, 2 pounds (0.9 kg) brown sugar, and 2 ounces (56.7 g) Instacure # 2 (containing salt, 6.25% sodium nitrite and 1% sodium nitrate) for every 100 pounds of green product (Graham et al., 2012). Hams were salted 2 d for every 1 lb of fresh product weight and went through a 15 d equalization process (7 ˚C) before exposure to warmer temperatures. The hams aged for approximately 6 mo at 23 ºC. After cure, hams were skinned, deboned, trimmed of intermuscular fat, and cut into 1.27 cm cubes. Cubes were then vacuum sealed and frozen for later use.

Garlic Juice Extraction

Monviso garlic was sourced from Christopher Ranch, Gilroy California. Garlic cloves were peeled and placed into an Omega J8003 juicer (1681 California Ave, Corona, CA 92881). The juice passed through two screens removing most of the large particulates. The extract was placed into 50 ml conical tubes and centrifuged at 3000 rpm for 40 min, separating the remaining solids and liquids. The liquid content was then removed and stored at room temperature between 20-22 ºC.

Mite Behavioral (Selectivity) Trials

Behavior trials were used to show the repellent effects of fresh garlic juice. Twenty adult female mites were placed in 8.9 cm petri dishes. Whatman™ 1827-082 Grade 934-AH Glass Fiber Filter Paper without Binder, Diameter: 8.2cm, Pore Size: 1.5µm covered the bottom of the petri dish and three adjoining linear 2.12 cm circles were drawn similar to procedures outlined previously (Abbar et al., 2016). To prevent mite escape, a thick continuous ring of petroleum jelly encircled the entire filter paper arena. Each plate received two ham cubes, one dipped in fresh garlic juice (FGJ) and one dipped in distilled water. Ham cubes were completely submerged and allowed to sit for 120 sec, then suspended for 60 sec. Cubes were then placed in opposing circles with mites placed in the middle circle. Plates were covered and placed in a growth chamber at 25º C and 75% RH. After two hrs, mite number alive, dead, and immobile on the ham surfaces and within each circle were enumerated. Each treatment was replicated five times.

Garlic Juice Direct Contact

Initial direct contact trials compared FGJ to no treatment control (NTC) and an equal volume of distilled water and linalool. Petri dish bottoms were covered with petroleum jelly encircled filter papers with a 2.54 cm circle traced in the middle of each. Samples were positioned at the center of the circle and allowed to wick into the filter paper for 60 sec. Ten adult female mites were then placed around the perimeter of the traced circle. One ham cube was placed at the center, and each treatment was placed in a separate growth chamber. Number of live, dead, and immobile mites were enumerated after 24 hrs. Higher volumes than 200 µl of water controls over-saturated the filter paper and resulted in death. A volume of 200 µl saturates the 2.54 cm circle that the mites must cross to reach the ham cube without excessive moisture.

To reduce the effects of inherent garlic juice viscosity, filter paper was used as a wicking mechanism to draw solution and ensure equal distribution of materials. Therefore, no standing or pooled materials could confound results. Mites were placed on the perimeter of the circle so they had to cross the filter paper to reach the ham cube. To minimize mite escape, a thick continuous ring of petroleum jelly encircled the entire filter paper. All treatments were placed into separate incubation chambers that were held at the same relative humidity (75%) and temperature of 25 ºC.

Garlic Juice Volatility

A noncontact design was used to examine if garlic juice volatiles influence mite mortality. Treatment groups consisted of control (NTC), 600 µl linalool, and 600 µl FGJ. All controls groups were replicated six times. For this assay, ten adult female mites and ham cubes were placed into a 3.81 cm petri dish with a thick petroleum jelly barrier sealed the filter paper covering the bottom. Dishes were then placed into a larger 8.9 cm petri dish. FGJ treatments and the linalool control were deposited around the perimeter of the larger dish. The smaller dish was covered with both filter paper and the lid to help retain volatiles. Assays were placed into separate growth chambers that were held at 25º C and 75% humidity.

Garlic Juice Freshness Titration

To test the long term effectiveness of garlic juice, garlic was juiced and aged for 0, 8, 24, 48, and 72-hour periods. Titrations of 25% GJ, 50% GJ, 75% GJ (diluted with distilled water), and 100% GJ was also added to see if garlic concentration played a role in overall efficacy. 200 µl of each concentration was used and the bottom of the petri dishes were covered with filter papers sealed with a thick ring of petroleum jelly. A 2.54 cm circle was traced in the middle of

each. Treatments and controls were deposited at the center of 2.54 cm circle and allowed to wick into the filter paper for 60 seconds. 10 adult female mites were placed around the perimeter of the traced circle. Then, one ham cube was positioned at the center, and each treatment was placed in individual growth chambers. Number of live, dead, and immobile mites were counted at 15, 30, 60, 120, 240, and 1440 min to compare mortality rates.

For all dilutions, equal volumes of 200 µl solution was deposited to minimize volume based variation among groups. Additionally, garlic was examined at different time points to obtain mortality rates. Garlic juice was applied fresh (0), 8, 24, 48, and 72 hours after juice extraction. Aged garlic was stored in a lighted room at an average of temp 21º C +/- 2º C, and all juice was derived from the same garlic distributer. Unused cloves were stored in a cool dry place for the duration of the assay. To evaluate the toxicity variation among the variant concentrations and juice age, additional time counts of 15 min, 30 min, 60 min, 120 min, 240 min, and 1440 min were added. Moreover, lethal concentration (LC) and lethal time (LT) was calculated with corresponding 95% confidence interval (CI) using Poloplus Software. Due to the labor-intensive inoculation and counting process, a base count of 15 min was the first plausible time point to begin each assay.

Results & Discussion

Results from the selectivity behavior experiment indicate that mites prefer food without fresh garlic juice. Figure 1 shows that after 2 hrs, no mites were detected on the surface of the meat cubes dipped in fresh juiced treatment. Control cubes dipped in distilled water possessed approximately 65% ($P<0.0001$) of the inoculated mite population. Remaining mites were located, but not on the surface of either the treatment or control cubes. These data suggest fresh

garlic juice is offensive to ham mites. The exact reason for this repellency is unknown. One possibility is that mites were somehow affected by volatiles through olfactory signalling. However, considering all inoculated mites were alive, the compounds were not toxic enough at the levels tested to produce mortality. Alternatively, more than 2 hr of exposure may be necessary to cause mortality in this choice test experimental design. Also, aside from factory-matched lid, no additional effort to retain volatiles was imposed. However, considering mites were able to avoid contact with garlic juice, contact exposure effects would also need to be determined.

Volatile application of essential oils (cardamom, cinnamon, clove, eucalyptus and jasmine) are utilized in controlling other mite species, such as the spotted spider mite (Tasnin & Khalequzzaman, 2016). More specifically, volatile efficacy against the ham mite has been shown for cinnamon and pine essential oils (Kim et al., 2004; Macchioni et al., 2002). Castan (2001) examined inhalation efficacy of numerous monoterpenes (terpene component found in fruit and plants) including linalool, eucalyptol, fenchone, menthone, and pulegone. The results indicated that mites can indeed be affected through volatile action of compounds derived from botanical sources. Garlic juice is also a botanical derivative; so volatile action is not unlikely.

Based on our initial design, it was not possible to conclude whether it was the garlic extract itself, or the resulting volatile compounds that led to reduced mite infestations on the meat cubes. Therefore, we developed studies to determine if direct contact exposure to FGJ would affect mites. Figure 2 shows inclusion of linalool resulted in an average of only 0.06 living mites per meat cube. Linalool is a natural terpine alcholol extracted from various plant species such as lavender or mint, and was used as a positive control (Castan, 2001). Addition of FGJ, on the other hand, resulted in an average of 0.2 mites per cubes, significantly lower than the

negative control, where nearly 100% survived. These results suggest fresh garlic juice may somehow affect mites through direct contact exposure, or from long-term exposure to volatile components. Figure 3 shows the effect of 24 hr exposure to garlic juice volatiles. To accomplish this, it was necessary to ensure direct contact did not occur so a dual petri-dish experimental design was employed using an additional filter paper and factory lid to prevent volatile escape. FGJ mortality after 24 hr exposure did not differ from no treatment controls (NTC), though the linalool positive control differed ($P < 0.0001$) from controls. These data support the aforementioned results that suggested FGJ may inhibit mite infestations through direct contact. However, volatiles also function as a natural repellent given that garlic juice prevented mite colonization on covered product.

According to Prowse et al. (2006), garlic constituents can act as enzyme inhibitors, and one of the main enzymes inhibited being acetylcholinesterase. This inhibition is accredited to allicin, diallyl disulphide and diallyl trisulphide (Singh & Singh, 1996; Bhatnagar-Thomas & Pal, 1974b). The polysulphide groups making up these compounds may be responsible for the repellency and eventual toxicity of garlic juice (Prowse et al., 2006). Polysulphide groups cross-link with thiol constituents of enzyme structure, leading to changes in protein shape, function, and eventually denaturation and inactivation (Halliwell & Gutteridge, 1999).

However, garlic is known for chemical instability among its constituents, including those thought to have insecticidal properties. Therefore, one of the main complications when using garlic is the consistency of garlic juice composition, shelf life, and the variation in efficacy of garlic derivatives due to inconstant enzymatic breakdown (Prowse et al., 2006). In addition, different orders, species, and sub-species life stages can react differently to garlic compounds.

This disparity often hinders the successful implementation of garlic-based compounds as feasible insecticidal products.

Due to variable response concerns and possibility that FGJ deteriorates on extraction (Prowse et al., 2006), a stability study was conducted where FGJ was applied after variable aging periods. Using a similar experimental design, FGJ was used at 100%, 75%, 50%, and 25% concentrations. Table 1 shows that concentrations of 100% and 75% fresh garlic juice were effective after approximately 60 min and 240 min, respectively, killing 99.3% of the mites added. As the concentration was reduced to 50%, the time necessary to kill 95% of the population increased to 1440 min. Likewise, FGJ diluted to 25% failed to achieve 90% or greater mortality even after a 1440 min, or 24 hr duration. The disparity of the time necessary to reach 95% efficacy shows FGJ works in a concentration dependent manner. LC, LT, and 95% CI values decreased as time increased, supporting the idea that relative toxicity increases with exposure time. LC_{50} values represent the concentration necessary to produce 50% mortality, and is an indicator of compound toxicity. As values decrease, it shows a lower concentration is necessary to kill relatively the same number of pest as a higher number. LT_{50} represents the time necessary to produce 50% mortality. Therefore, lower LT_{50} values represent less time necessary to kill the pest population. For both, a supplemental 95% confidence interval (CI) represents the accuracy or variation within the given population that lays within 95% of the mean represented in a standard bell curve. These interpretations apply to all the data Tables.

Table 2 shows the effect of 8 hr ageing on FGJ efficacy. Aging before bioassay reduced mortality rate, but still yielded complete mortality for 100% juice. The 75% juice was only 96% effective after 240 min, and did not increase in mortality after the end of the trial. Meanwhile, the 50% garlic juice was only able to reach 84% mortality after 1440 min exposure. This pattern

held true for the 25% juice, which only killed 67% when concluded. Again, LC, LT, and 95% CI values decreased with exposure, but were higher than those portrayed in Table. 1 for FGJ, meaning that toxicity appears to be decreasing as juice age increases.

Table 3 shows the effect of a 24 hr age on FGJ efficacy. For the 100% garlic juice sample, initial 15 min and 30 min mortality was 15% greater than the 8hr, but lower for the remaining time points until still reaching 100% at 1440 min. The 75% juice yielded 97.3% mortality after the 24 hr period. Neither the 50% or 25% titrations were as effective as the 8-hour, only reaching 76% and 57% mortality in completion. Once again, mortality rate retardation indicates that efficacy is concentration- and freshness-dependent**.** Again, supporting LC, LT, and 95% CI values decreased with exposure, but were higher than those portrayed in Table. 2, meaning that relative toxicity is still decreasing with juice age.

Continuing the pattern of previous results, the 48 hr garlic juice depicted in (Table. 4) was less effective than fresher versions. The 100% juice did produce 99.3% mortality after the 1440min duration, but the effectiveness was slower than previous concentrations. Moreover, the 75% juice reached a final mortality of 94%, which is lower than garlic age treatments concentrations at the same time point. 50% juice only reached 63% mortality after the full 1440min. Moreover, 26% was the maximum mortality expressed in the 25% concentration, lower than fresher juices. Following the same trend, supporting LC, LT, and 95% CI values decreased with exposure, but were overall higher than those portrayed (Table. 3), meaning that toxicity is continuously decreasing with age.

Shown in Table. 5, the highest mortality achieved for the 72-hour juice was 76% observed in the 100% concentration. Moreover, the 75% juice killed just over 60% of mites on

average. Continuing the same pattern, both the 50% and 25% garlic juice concentrations displayed around 40% total mortality. Finally, supporting LC, LT, and 95% CI values decreased with exposure, but were overall higher at every time point than any of the aforementioned results, meaning that toxicity is at the lowest among all aged garlic juice groups. Because complete efficacy was not achieved, no further ages point were examined.

Results of these studies show that as garlic juice concentration decreases or age increases, the overall efficacy of FGJ killing mites is retarded. Though the exact mechanism for this loss of efficacy is not known at present, it is probably due to breakdown of some constituent (Barnard, 1957). Cañizares et al., (2004) showed the thermal degradation of allicin is highly dependent in temperature and when held just above room temperature (26º C) only persists a few days. Furthermore, Brodnitz et al. (1971) showed that the active ingredient allicin when stored at 20 °C for 24 hr decomposed almost entirely to diallyl disulfide, diallyl sulfide, and diallyl trisulfide, and SO_2 due to thermal instability. Curiously, in this study garlic treatments were juiced and stored at similar temperatures (20-22º C), potentially hastening enzymatic processes. In our studies, mite incubation chambers were held at 25º C, most likely accelerating degradation. This could explain the reduction in effectiveness between the fresh and stored garlic.

Incorporation of LC_{50}, LT_{50}, and 95% CI provides insight to the relative garlic toxicity. Treatments represented by lower numbers were deemed more toxic because less time was necessary for the substrate to produce 50% mortality. Supporting the mortality results, LC and LT values show a declining trend in toxicity as juice was aged and concentration reduced. Juices of the same concentration but older ages, were consistently less toxic (higher values) than fresher counterparts. In addition, time needed to produce full mortality was arrested, for undiluted

treatments after 48-hours. Once again, indicating a compositional breakdown within the juice as age is extended.

In an effort to extend garlic juice functionality, 1mM solutions of antioxidants or metal chelators including: ascorbic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid (EDTA), and ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) were examined. In addition, pH 7 (neutralized with potassium hydroxide) garlic juice was also implemented. Ascorbic acid is a natural plant metabolite that functions as an antioxidant. In addition, BHA and BHT were also examined because they are commonly thought to be functionally stronger antioxidants than ascorbic acid. EDTA and EGTA were tested to see if toxicity was affected via oxidation catalyzed by metal ions. pH neutralization was tested because garlic breakdown is thought to be due to natural enzymatic processes (Carson, 1987).

The experiment was run as a RCBD with three plates (10 mites/ plate) per treatment. As with previous studies, the number alive, dead, and immobile was counted after 24-hours. Results show that toxicity was not extended by any treatment process. Moreover, pH neutralization drastically reduced the toxicity to the point of equaling the water control. This result indicated the functionality is related to the natural enzymatic processes of garlic decomposition. We know the unique flavor and aroma found in allium species is due to naturally occurring enzymatic process that are pH and thermally regulated (Carson, 1987). In addition, the enzymatic intermediates are thought to contain the insecticidal properties. So, if enzymatic process are inhibited, so may be the production of insecticidal components. Therefore, it is likely that pH neutralization also neutralized functionality through prevention of key intermediates. However, unfortunately, the functionality is not likely easily altered through incorporation of food grade

antioxidants or metal chelating agents. Rather, functionality extension will need to be through constituent isolation and enzymatic stabilization.

Conclusions

Fresh garlic juice is effective in producing ham mite mortality. Though the exact mechanism for efficacy appears to be related to direct exposure to the juice. Yet, the ingredient responsible for this increased mortality is labile and the overall effectiveness declines with time. Additional work is warranted to determine the utility of using garlic as a safe and efficient means of control ham mite populations.

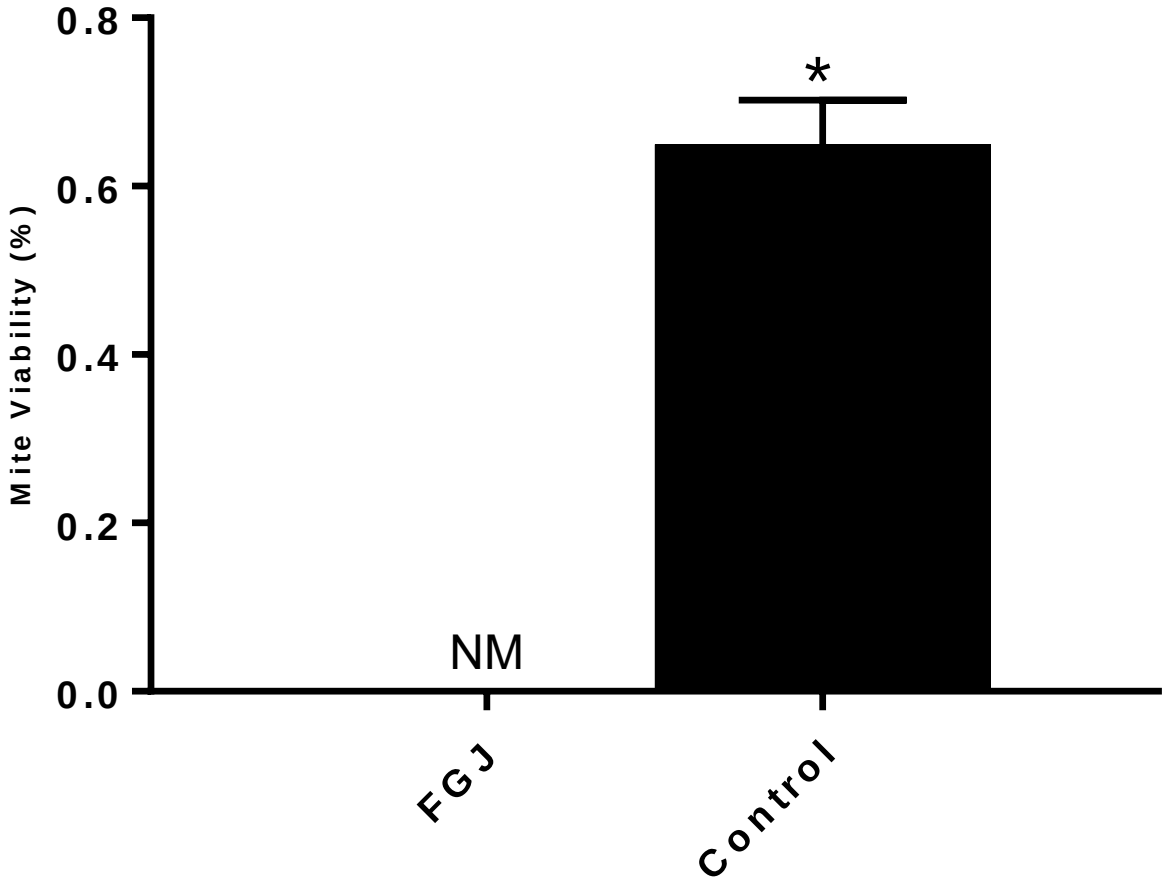

Figure 1. Effect of fresh garlic juice (FGJ) on mite viability. Ham cubes were dipped in water or FBJ, drip-dried and inoculated with mites. After 2 hrs, mites were enumerated and reported as % viable. Asterisks indicate a difference ($P < 0.0001$) NM = No mites detected.

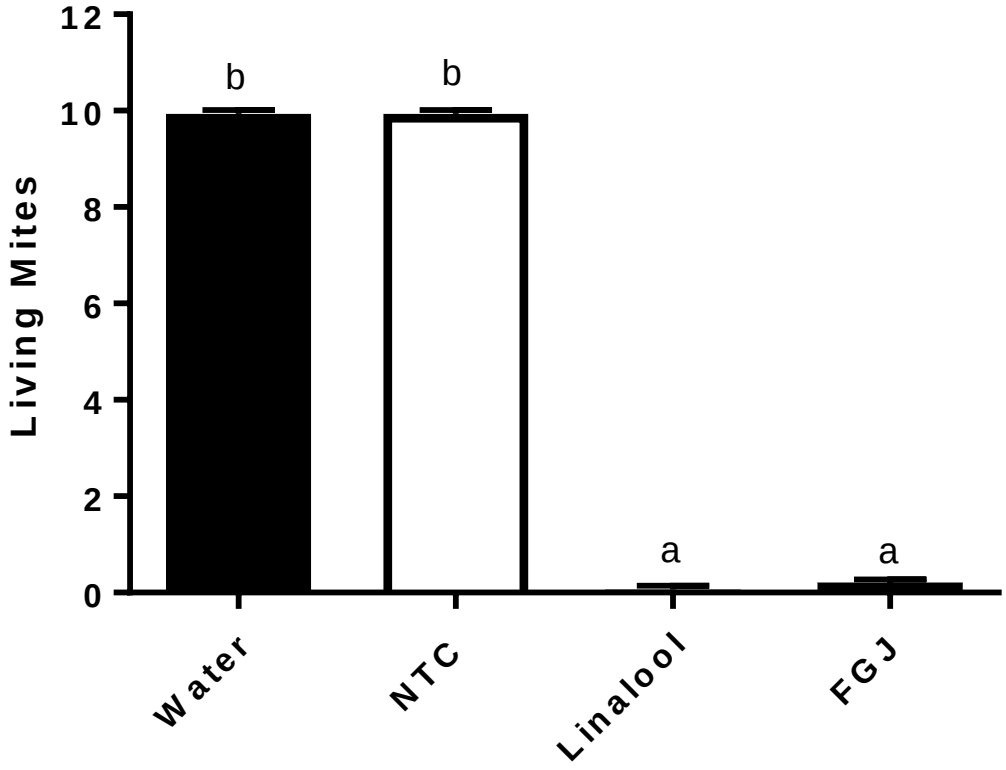

Figure 2. Effect of direct exposure on mite number. Fresh garlic juice FGJ was compared to a positive control of linalool (Lin-C) and negative controls of distilled water and no treatment control (NTC). Corresponding letters indicate no difference and ($P < 0.0001$). 10 Mites inoculated.

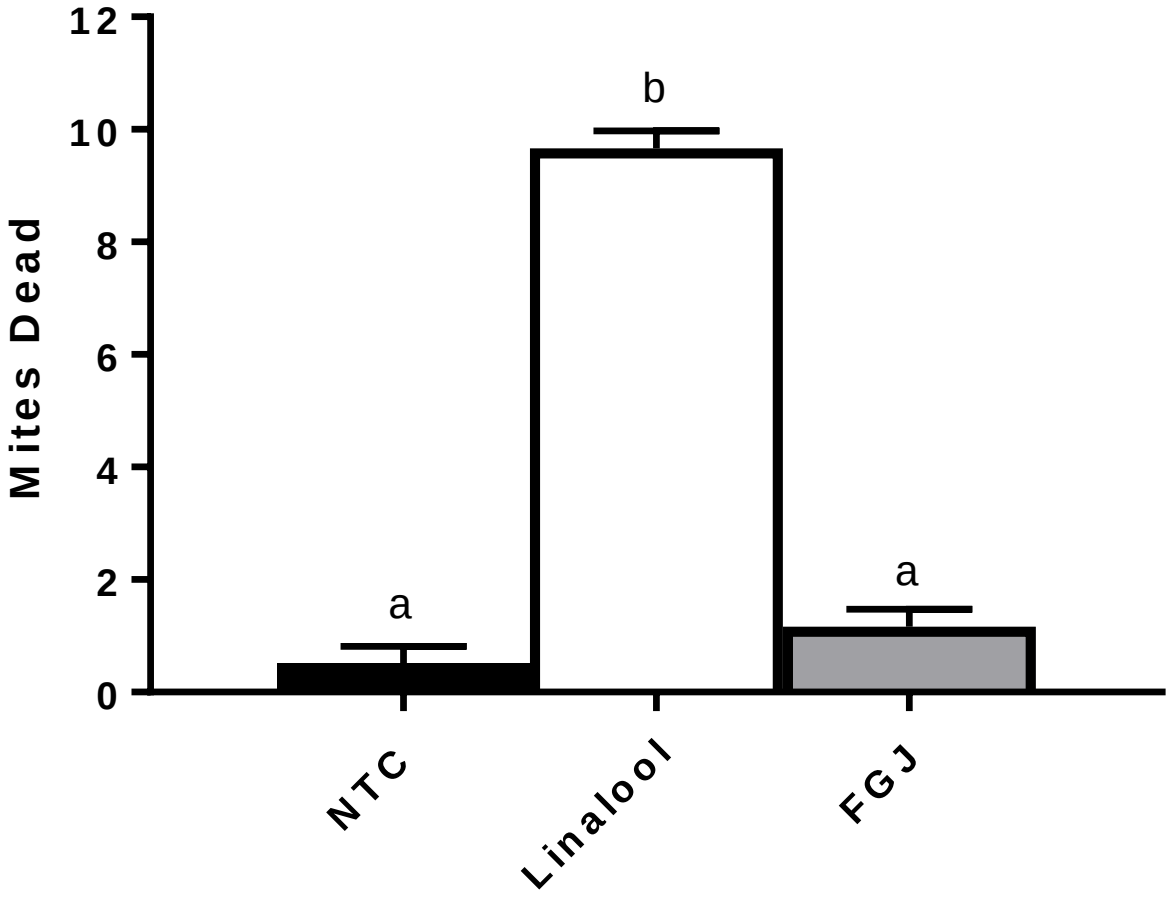

Figure 3. Effect of volatile fresh garlic juice (FGJ) compounds on mite mortality. FGJ was compared to a positive control of linalool and negative control of no treatment control (NTC). Corresponding letters indicate no difference and ($P < 0.0001$).

Table 1. Effect of fresh garlic juice concentration and exposure time on mite mortality. Treatment mean mortality and standard error (SE) are given for each concentration and time point. Lethal concentration (LC, in % undiluted juice), lethal time (LT, in min), along with 95% confidence interval (CI) values are also calculated where applicable based on mortality data, and are defined identically in all Tables. NA = not available.

	Fresh Garlic Juice % Mortality						
FGJ	15 min	30 min	60 min	120 min	240 min	1440 min	LT_{50} 95% CI
100%	37.3	93.3	99.3	99.3	100.0	100.0	NA
SE	7.7	2.5	0.7	0.6	0.0	0.0	NA
75%	3.3	75.3	94.7	96.7	99.3	100.0	NA
SE	1.6	5.8	1.9	1.6	0.7	0.0	NA
50%	0.0	2.7	57	73	85	95	206
SE	0.0	1.5	3.2	4.0	3.5	1.9	92-349
25%	0.0	0.0	2.0	3.3	14	87	901
SE	0.0	0.0	1.1	1.3	3.5	3.6	819-995
LC_{50}	NA	71	50	45	38	NA	
95% CI	NA	67-74	44-55	42-48	35-41	NA	

Table 2. Effect of stored (8 hr) garlic juice concentration and exposure time on mite mortality. Treatment mean mortality and standard error (SE) are given for each concentration and time point, with toxicity indices as described in Table 1. NA = not available.

	8 hr Garlic Juice % Mortality						
GJ	15 min	30 min	60 min	120 min	240 min	1440 min	LT_{50} 95% CI
100%	13.3	30.7	80.7	94.7	98.7	99.3	NA
SE	3.7	5.0	4.9	2.4	0.9	0.7	NA
75%	0.0	6.7	38.7	82.0	96.0	96.7	NA
SE	0.0	2.1	6.0	3.8	1.3	1.3	NA
50%	0.0	0.7	16.0	28.0	40.7	84.0	734
SE	0.0	0.7	2.4	3.7	3.0	5.2	624-875
25%	0.0	0.0	2.0	10.7	18.7	67.3	1104
SE	0.0	0.0	1.1	1.8	3.4	7.2	984-1254
LC_{50}	NA	NA	79	59	48	22	
95% CI	NA	NA	75-89	55-62	45-52	9.5-30	

Table 3. Effect of stored (24 hr) garlic juice concentration and exposure time count for mite mortality. Treatment mean mortality and standard error (SE) are given for each concentration and time point, with toxicity indices as described in Table 1. NA = not available.

	24 hr Garlic Juice % Mortality						
GJ	15 min	30 min	60 min	120 min	240 min	1440 min	LT_{50} 95% CI
100%	26.7	45.3	72.0	94.7	96.7	100.0	37
SE	7.9	10.5	8.9	1.9	1.3	0.0	14-55
75%	7.3	29.3	44.7	78.0	82.0	97.3	128.6
SE	2.4	6.4	10.4	7.4	6.8	1.5	NA
50%	2.0	7.3	28.0	41.3	55.3	76.7	648
SE	1.1	1.8	8.7	11.0	8.8	6.5	457-959
25%	0.0	1.3	6.0	21.3	30.0	57.3	
SE	0.0	0.9	2.5	6.9	7.9	8.5	
LC_{50}	NA	NA	77	60	48	22	
95% CI	NA	NA	67-89	50-68	45-52	9.5-30	

Table 4. Effect of stored (48 hr) garlic juice concentration and exposure time on mite mortality. Treatment mean mortality and standard error (SE) are given for each concentration and time point, with toxicity indices as described in Table 1. NA = not available.

	48 hr Garlic Juice % Mortality						
GJ	15 min	30 min	60 min	120 min	240 min	1440 min	LT 50 95% CI
100%	4.7	20.7	60.7	84.0	92.0	99.3	NA
SE	1.9	6.9	0.7	5.8	3.1	0.7	NA
75%	0.0	6.7	21.3	54.0	60.7	94.0	416
SE	0.0	2.7	5.3	6.1	6.9	2.4	311-569
50%	0.0	1.3	6.7	12.7	26.0	63.3	1144
SE	0.0	1.3	2.3	4.4	6.8	4.4	966-1397
25%	0.0	0.0	0.7	1.3	3.3	26.0	1951
SE	0.0	0.0	0.7	0.9	1.9	3.8	1717-2297
LC_{50}	NA	NA	92	74.7	67	41	--
95% CI	NA	NA	87-99	70.1-72.6	62-72	37-44	--

Table 5. Effect of stored (48 hr) garlic juice concentration and exposure time on mite mortality. Treatment mean mortality and standard error (SE) are given for each concentration and time point, with toxicity indices as described in Table 1. NA = not available..

	72 hr Garlic Juice % Mortality						
GJ	15 min	30 min	60 min	120 min	240 min	1440 min	LT_{50} 95% CI
100%	0.0	2.0	32.7	38.7	47.3	76.7	732
SE	0.0	1.4	5.5	6.0	4.9	6.4	569-975
75%	0.0	0.7	12.0	22.0	34.7	60.7	NA
SE	0.0	0.7	2.8	5.2	5.5	4.7	NA
50%	0.0	0.0	0.7	8.7	19.3	42.0	1521
SE	0.0	0.0	0.7	3.1	4.3	5.5	1287-1876
25%	0.0	0.0	0.0	4.0	12.0	40.7	1542
SE	0.0	0.0	0.0	1.9	4.9	6.9	1310-1895
LC_{50}	NA	NA	NA	NA	NA	NA	
95% CI	NA	NA	NA	NA	NA	NA	

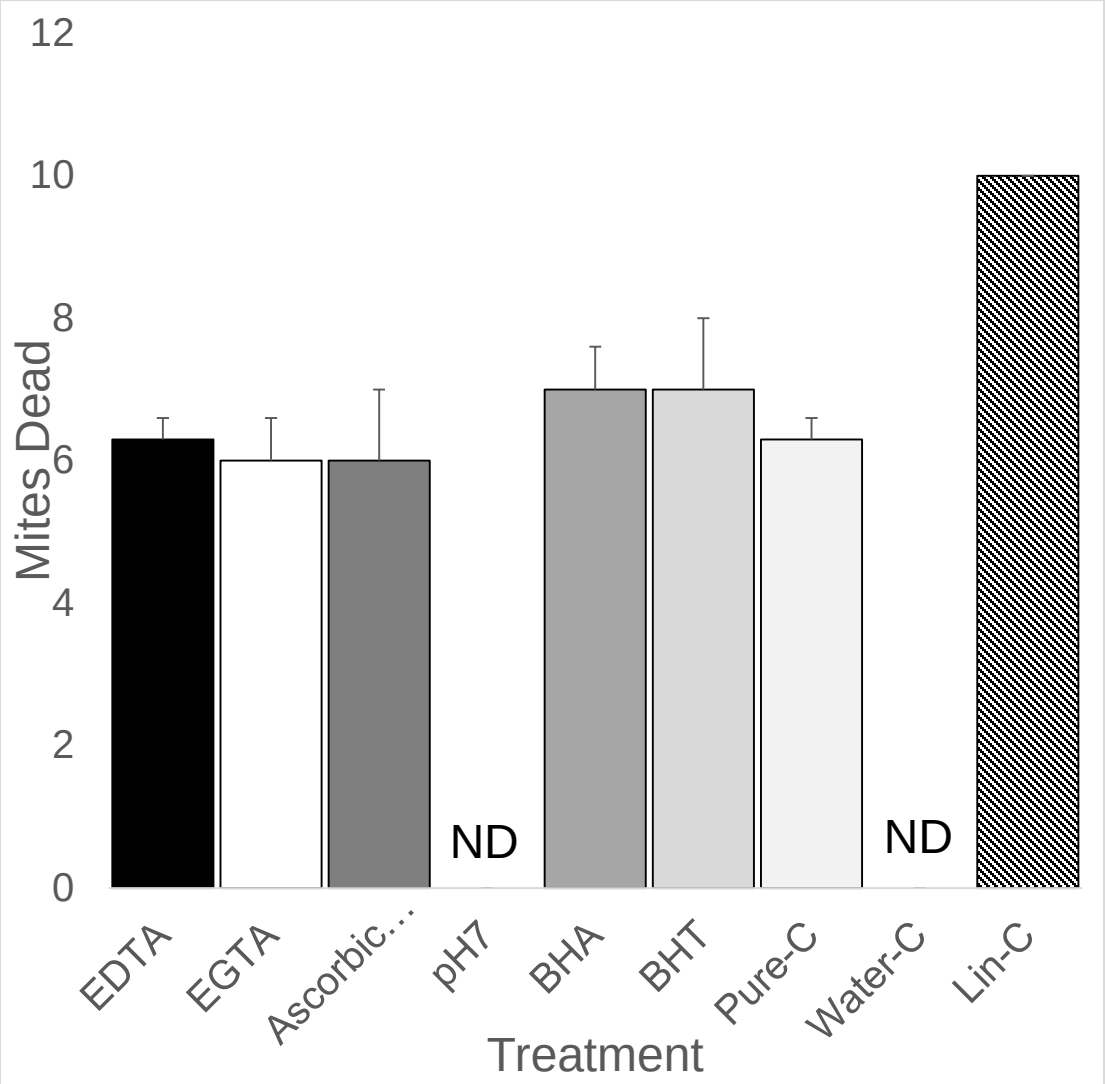

Figure 4. Effect of various amendments on garlic juice ortality to ham mites. The **mean** number of living mites on the cube surface using a 24-hour exposure time with 10 mites inoculated per plate. ($P < 0.0001$). ND= No death.

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Chapter 3. Future Directions

Although garlic juice proved effective on adult ham mites, many pieces of the puzzle are lacking before it can be implemented as a natural ham mite control measure. Efficacy in hatch rate studies would indicate if garlic juice can be considered a complete ham mite control alternative. In many pest species, egg stages can be more tolerant to insecticide applications. If the same is true with ham mite eggs, the relatively short term effect of garlic juice could mean increased monitoring and number of applications would be necessary. This in turn increases relative cost and brings to question flavor change differences among applications.

A multitude of factors affects final ham quality and when testing any compound, it is essential that minimal quality defects occur. This is especially a concern with compounds known for strong flavor profiles, such as garlic and previously examined botanicals. Therefore, further studies on the long term flavor effects of garlic oils on country hams need to be implemented. Ideally, this would be completed through a full scale model where hams go from start to finish. Furthermore, it would be interesting to test if application method and frequency of application affects mite densities. For example, if juice is applied every two weeks versus once a month or whole ham dip versus a topical spray would be helpful. A simple taste panel could be assembled to gather this information and help shed light on if further garlic control measures should be pursued on a ham quality basis.

In addition, in order for the process to be cost effective, commercialization of consistent garlic juice product would be necessary. In order to be seriously considered as a viable control method, it is unlikely that a highly variable product such as garlic juice would be appropriate. Being comprised, pinpointing the effective acaracidal component(s) would help determine the

reason for garlic efficacy. If the component is commercially synthesized, it could be cheaper, more effective, and less labor intensive than whole garlic juice applications.