Journal of Stored Products Research 79 (2018) 73–78

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Acaricidal effects of fresh garlic juice on adult ham mite, *Tyrophagus putrescentiae* (Schrank)

R.H. Preisser III [a], T.D. Anderson [b], F. Demares [c], J.R. Bloomquist [c, *], D.E. Gerrard [a]

[a] Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA, 24061, USA
[b] Department of Entomology, University of Nebraska, Lincoln, NE, 68583, USA
[c] Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL, 32610, USA

ARTICLE INFO

Article history:
Received 4 August 2018
Received in revised form
18 September 2018
Accepted 20 September 2018

Keywords:
Acaricide
Allium sativum
Toxicity
Natural product

ABSTRACT

Ham mites, *Tyrophagus putrescentiae* (Schrank), are a common pest of dry cured meat products and cause devastating effects on product quality. Methyl bromide, a chemical fumigant used to control mite populations, is no longer be available due to regulatory action. Therefore, it is essential to identify potential alternatives. Garlic (*Allium sativum*) extracts or chemical components show toxicity to the northern fowl mite, mosquitoes, nematodes, and aphids. Thus, we explored the efficacy of garlic juice in controlling *T. putrescentiae*. Using a choice test experimental design, approximately 50% of inoculated mites colonized control ham cubes, while no mites remained on cubes dipped in fresh garlic juice. Garlic was ineffective when examined for volatile efficacy, but was toxic in direct contact assays, showing time- and concentration-dependent lethality. Fresh garlic juice at 50–100% strength showed ≥95% mortality in 24 h bioassays, well within the range for acceptable commercial efficacy. However, as fresh garlic juice aged up to 72 h, efficacy was significantly reduced, particularly when diluted with water. Thus, garlic juice acted as a short-term repellent and toxicant in contact models, but application is time sensitive. Nonetheless, it shows potential utility for control of the ham mite.

1. Introduction

The ham mite (*Tyrophagus putrescentiae*) is a common pest of cured hams, with infestations imparting a pungent smell and powdery or dusty appearance (Nayak, 2006). Control of *T. putrescentiae* has long employed the use of methyl bromide as a fumigant (Nayak, 2006). However, damage to atmospheric ozone has since rendered methyl bromide obsolete under the Montreal Protocol (USDA, 2000). Therefore, effective alternatives 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 for control of *T. putrescentiae* include: other fumigants, food-safe coatings, and natural products and temperature (Garcia, 2004; Nayak, 2006). Previous studies have shown the efficacy of naturally-derived substances such as pine (Macchioni et al., 2002), cinnamon (Kim et al., 2004), peppermint (Park et al., 2014), and thyme (Jeong et al., 2008) for control of ham mite.

Garlic, *Allium sativum* (L.), is another plant with pest control potential. It contains organosulfur compounds that are naturally found in related plants, such as onions and leeks, but are generally more concentrated in garlic (Awais et al., 2014). Allicin is an important chemical component of garlic, contributing to the unique smell and flavor, which is derived through enzymatic breakdown of alliin by alliinase when plant cell walls are disrupted (Awais et al., 2014). Allicin is a reactive compound and its production provides a natural defense system against pests and pathogens (Awais et al., 2014). Moreover, a commercial mixture of garlic-based organosulfur components is known to be toxic to nematodes (Awais et al., 2014). Garlic juice has been shown to have insecticidal activity against two dipteran pests, *Delia radicum* (L.) and *Musca domestica* (L.) similar to that of the organophosphate, chlorfenvinphos (Prowse et al., 2006). Garlic is also toxic to the northern fowl mite by topical application to laying hens (Birrenkott et al., 2000). In terms of mode of action, allicin use as a molluscicide resulted in reduced activities of acetylcholinesterase, a known insecticide target site, as well as other enzymes (Singh and Singh, 1996).

Based on the aforementioned studies, garlic was investigated as an alternative treatment for control of the ham mite in this study.

* Corresponding author. Emerging Pathogens Institute, Department of Entomology and Nematology, 2055 Mowry Road, PO Box 100009, University of Florida, Gainesville, FL, 32601, USA.
E-mail address: jbquist@epi.ufl.edu (J.R. Bloomquist).

https://doi.org/10.1016/j.jspr.2018.09.004
0022-474X/

The central hypothesis was that ham mites could be controlled by incorporating generally recognized as safe (GRAS) compounds, such as garlic juice, on the surface of hams. Experiments outlined herein compared the acaricidal effectiveness of GRAS compounds within a controlled and industry-relevant environment.

2. Materials and methods

2.1. Mite colony rearing

Tyrophagus putrescentiae specimens and a diet recipe were obtained from Dr. Thomas Phillips (Kansas State University, Manhattan, Kansas, USA) and raised in 946 ml glass jars sealed with filter paper to allow for gas exchange. Colonies were fed a specialized diet bi-weekly and housed in a growth chamber held at approximately 25 °C (+/− 5 °C) and 50% (±10%) r.h. Holding chambers were housed in a separately ventilated room from treatment chambers to prevent exposure of the colonies to any volatiles. Diets consisted of 320 g Beneful™ (Purina Animal Nutrition LLC, Minneapolis MN) 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, and 7.9 ml of 15% methylparaben in 95% ethanol as a preservative. Water and dog food were placed into a 3.8-L container and heated to render the dog food. Once the wet portion was simmering, remaining ingredients were added, thoroughly mixing between each. Colony jars were filled with dry dog food approximately half way, and the liquid portion was distributed among the jars. After distribution, the wet and dry constituents were mixed thoroughly, allowed to cool for approximately 1 h, and inoculated with three tablespoons of mites.

2.2. Ham cube sample preparation and garlic juice extraction

Salt cured hams were fabricated in the Virginia Tech Meat Center. Hams were processed using the 8-2-2 recipe, which consists of 8 lbs (3.6 kg) salt, 2 lbs (0.9 kg) brown sugar, and 2 oz (56.7 g) Instacure # 2 (containing salt, 6.25% sodium nitrite and 1% sodium nitrate) for every 100 lbs (45.5 kg) of green product (Graham et al., 2012). Hams were salted 2 d for every 1 lb (454 g) of fresh product weight and were exposed to a 15 d equalization process at 7 °C. The hams were then aged for approximately 6 mo at 23 °C. 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.

Monviso garlic (Christopher Ranch, Gilroy, California, USA) cloves were peeled and placed into a J8003 juicer (Omega, 1681 California Ave, Corona, CA 92881). Blended garlic was passed through two screens to remove large particulates. Extracts were then placed in 50 ml conical tubes and centrifuged for 40 min at 3000 rpm to separate the remaining solids. Supernatants were removed and stored at room temperature (20–22 °C).

2.3. Behavioral choice tests

Behavioral trials were used to assess the repellent effects of fresh garlic juice in a choice paradigm. The behavioral arena (Fig. 1A) consisted of a 8.9 cm petri dish lined with glass fiber filter paper (Whatman™ 1827-082 Grade 934-AH, 1.5 μm pore size). Three adjoining 2.12 cm circles arranged in a line were drawn on the paper, similar to procedures described previously (Abbar et al., 2016). To prevent mite escape, a thick continuous ring of petroleum jelly encircled the entire filter paper arena. Each petri dish received two ham cubes, one dipped in fresh garlic juice (FGJ) and one dipped in distilled water. Ham cubes were completely submerged for 120 s, then suspended and allowed to dry for 60 s. Treated cubes were then placed within the left- and right-hand circles, and 20 mites placed in the middle circle. Petri dishes were covered and placed in a growth chamber at 25 °C and 75% r.h. After two hr, the number of mites alive, dead, and immobile on the ham surfaces and within each circle were enumerated. Each treatment was replicated five times.

2.4. Volatility studies

A noncontact design was used to examine if garlic juice volatiles influenced mite mortality. Treatment groups consisted of no treatment controls, 600 μl linalool, and 600 μl FGJ. Linalool is a natural terpene alcohol extracted from various plant species such as lavender or mint (PubChem, 2018) having antimicrobial and insecticidal properties (Beier et al., 2014), and was used as a positive control. All control groups were replicated six times. For the assay, ten adult female mites and ham cubes were placed into a 3.8 cm petri dish and a petroleum jelly barrier sealed the filter paper covering the bottom (Fig. 1B). 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 25 °C and 75% r.h.

2.5. Direct contact studies

Direct contact trials compared FGJ to an equal volume of distilled water (negative control) and linalool (positive control). A Petri dish was lined with a filter paper having a 2.54 cm circle traced in the middle (Fig. 1C). Treatments (200 μl) were applied at the center of the circle and allowed to wick into the filter paper for 60 s. Ten adult female mites were then placed around the outer perimeter of the traced circle. An untreated ham cube was placed at the center, forcing the mites to cross the treated barrier to reach it. Mite escape from the arena was prevented by an outer ring of petroleum jelly and each treatment dish was placed in a separate growth chamber held at the same r.h. (75%) and temperature of 25 °C. The number of live (crawling normally), dead (not moving at all), and immobile (legs have movement, but unable to crawl) mites were enumerated via microscopic examination after 24 h.

2.6. Garlic juice dilution and aging studies

To test the long-term effectiveness of FGJ as a miticide, garlic was juiced and aged for 0, 8, 24, 48, and 72 h before mite exposure in the direct contact assay (Fig. 1C). It was tested at 100% strength or diluted with distilled water to concentrations of 75, 50, and 25% final strength. A 200 μl treatment of aged garlic juice was deposited at the center of the 2.54 cm circle and the assay conducted as described above for the direct contact studies. Number of live, dead, and immobile mites were counted at 15, 30, 60, 120, 240, and 1440 min to compare mortality at different exposure times. Due to the labor-intensive inoculation and counting process, an initial assessment of toxicity at 15 min was the first plausible time point for toxicity evaluation.

2.7. Statistical analyses

Tests were replicated 5 times with 10 mites per replicate for calculation of mean and SEM, and treatments were then subjected to *t*-test or ANOVA, as appropriate, using Prism™ 7 (GraphPad, San Diego, CA, USA). Survival of mites exposed to aged garlic juice was analyzed with Kaplan-Meier global analysis and pairwise comparisons using SPSS 25 (IBM Statistics). Lethal concentration to kill 50%