ABSTRACT

Diaphorina citri is a vector of the harmful citrus greening disease huanglongbing (HLB), which is caused by bacteria in the genus Candidatus Liberibacter. Jugglone is a natural chemical produced by walnut trees that has herbicidal, insecticidal, and bactericidal properties. This study tested the effects of jugglone on the gut microbiota of D. citri. The digestive tracts of fifteen individuals were extracted and plated, producing two different kinds of bacteria and five different types of fungi. Ten individuals were crushed whole and plated for comparison. Each unique bacterial and fungal colony was transferred to a plate with three wells: one for a positive control (erythromycin for bacteria; tea tree oil for fungi), one for negative control (water), and one for the experimental treatment (jugglone). Results were taken by measuring the radius of inhibition around each well. Both bacteria were inhibited by juglone, though not as strongly as they were by erythromycin. Two of the fungi were actually better inhibited by juglone than by the positive control, while the third one was not inhibited by juglone at all.

INTRODUCTION

The Asian citrus psyllid (ACP), Diaphorina citri (Figure 1), is an agricultural pest of citrus trees. It is particularly important because it is a vector of the citrus greening disease huanglongbing (HLB), which is caused by bacteria in the genus Candidatus Liberibacter. These bacteria reside in the phloem of their host tree, and the disease is spread when a psyllid feeds on an infected tree then flies to and feeds on a non-infected tree (CDFA, 2017). This disease has cost the citrus industry in Florida $4.5 billion in lost revenue between 2006 and 2011 (Hodges and Spaans, 2012). There is a method of biocontrol currently being applied to deal with the problem of ACP spreading HLB, and it involves using a natural predator of the ACP, Zomaria radialis, which is a wasp that parasitizes ACP specifically. A female wasp will oviposit one or two eggs underneath an ACP nymph, and the wasp larva feeds on the nymph as they grow, eventually consuming all of the nymph’s body contents, pupating, and flying away as an adult (Qureshi and Stansly, 2010). This method of biocontrol has had varying effects. In Florida, parasitism of ACP by T. radiata averaged 50% in the fall of 2009, while in Isabela, Puerto Rico, parasitic rates generally exceeded 50% and averaged 70% (Qureshi and Stansly, 2010).

RESULTS

There were two strains of bacteria found, a yellow one (Figure 5) and a white one (Figure 6). They were found on both, red and black plates, and they were always found together (Table 1). Two of the red plates had no bacterial growth (Table 1). The bacterial control plate (Figure 7) showed a 5.0cm radius of inhibition around the positive control well and a 0.5cm radius of inhibition around the experimental well (Table 2). The experimental plate with the yellow bacteria (Figure 8) showed a 2.1cm radius of inhibition around the positive control well and a 0.7cm radius of inhibition around the experimental well (Table 2). The experimental plate with the white bacteria (Figure 9) showed a 1.5cm radius of inhibition around the positive control well and a 0.7cm radius of inhibition around the experimental well (Table 2) and a 0.7cm radius of inhibition around the experimental well (Table 2). At expected, all three plates showed no inhibition around the negative control well (Table 2). There were three different types of fungus found: yellow, black, and dark green. All three fungus were found on both the red and black plates. Two of the black plates and two of the red plates had no fungal growth (Table 1). The fungal control plate (Figure 10) showed a 1.4cm radius of inhibition around the positive control well and a 1.4cm radius of inhibition around the experimental well (Table 3). The experimental plate with the yellow fungus (Figure 11) showed a 0.4cm radius of inhibition around the positive control well and a 0.6cm radius of inhibition around the experimental well (Table 3). As expected, all four plates showed no inhibition around the negative control well (Table 3).

METHODS AND MATERIALS

To test the effect of juglone on the microbiota of the ACP, fifteen individual ACP from a sterile environment were dissected in order to extract the digestive tract. The digestive tracts were crushed and streaked onto Luria Broth (LB) agar plates using a sterile loop. These plates were labeled the "red" plates. From the same environment, ten more ACP were taken, crushed, and streaked onto LB plates, which were labeled the "black" plates. These plates were left alone for a week, allowing any resulting bacterial or fungal colonies to mature. After four days of growth, however, some of the plates with large bacterial colonies had to be placed in a refrigerator in order to slow their growth. At the end of the week, a sample of each unique bacterial and fungal colony from both sets of plates was transferred onto new plates. The bacterial colonies were kept on LB agar plates, while the fungal colonies were transferred to Potato Dextrose Agar (PDA) plates, which were incubated at 30°C for 48 hours. The PDA plates received the same treatment as the LB plates, except the positive control was tea tree oil, instead of erythromycin, because of its antifungal properties (Peèiulytë, 2005). A control plate was also made using Pleurotus djamor as the fungal agent. These plates were allowed to develop for three days, after which the results were taken.

DISCUSSION

Only two types of bacteria were found in the gut of D. citri, and the lack of any other types of bacteria on the black plates, which had a whole ACP crushed on them, indicates that these bacteria come from the gut. Jugglone did inhibit the growth of these bacteria, but it was not as significant in its inhibition as erythromycin, the positive control. A higher concentration of juglone could be more effective, and further research would need to be done to test this.

LITERATURE CITED


