

Assessment of Variation in Annonaceous Acetogenin Activity in Pawpaw (*Asimina triloba*) Cultivars

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Introduction

- The North American pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit in the initial stages of commercial production in the United States (Pomper and Layne, 2005; Fig. 1).
- Annonaceous acetogenins are fatty acid derivatives found in the Annonaceae family, which includes both the genera *Annona* and *Asimina*. (Alali, et al., 1999).
- Botanical insecticides are attractive alternatives to synthetic chemical insecticides for pest management of crops because they pose little threat to the environment or to human health; however, few botanicals are currently used in agriculture (Isman, 2006).
- Some acetogenin compounds are potent inhibitors of complex I in the mitochondrial respiratory chain and display anti-tumor and insecticidal properties (Alali et al., 1999).
- Previous studies have identified asimicin and bullatacin (Fig. 6 and 7) along with over 40 other bioactive acetogenins and other compounds in the crude extracts of twigs, unripe fruits, seeds, root, bark, and ripe fruit of pawpaw (Pomper et al., 2007)(Fig. 8).
- Fruit biomass represents a potentially larger and a more cost effective source than twigs for extractable acetogenin compounds for use as a botanical pesticide.
- Acetogenin activity can vary with pawpaw cultivars (Pomper et al., 2007). Identification of pawpaw cultivars displaying a high acetogenin activity would be beneficial for growers wishing to grow pawpaw as a source of these compounds.
- Previously, only a few pawpaw cultivars had undergone assessment of their acetogenin activity.

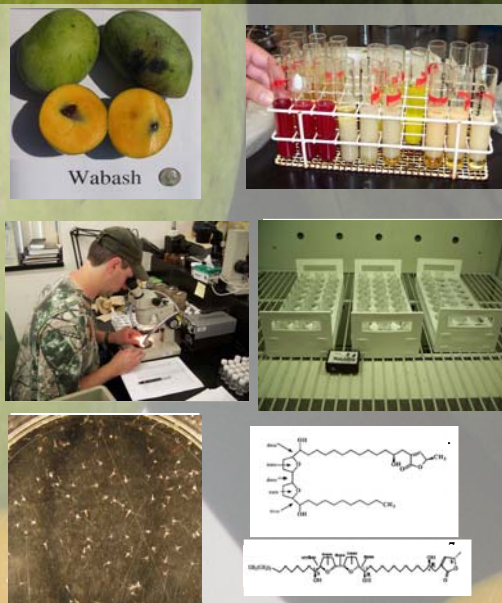


Fig. 1: A 'Wabash' fruit; Fig. 2: extracts of pawpaw and other fruits; Fig. 3: Counting brine shrimp; Fig. 4: vials containing brine shrimp and fruit extracts in growth chamber; Fig. 5: brine shrimp in petri dish; Fig. 6: Asimicin structure; Fig. 7: Bullatacin structure

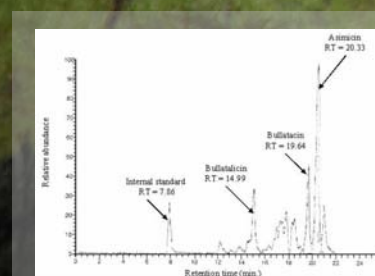


Figure 8: Chromatogram of pawpaw fruit extract

Results

- All 16 genotypes tested displayed acetogenin activity.
- The greatest differences were detected at a concentration of 10 ppm.
- The genotypes Middletown, NC-1, Taylor, Taytwo, 2-10, 3-11, and 11-13 contained a high amount of acetogenin activity, killing over 90% of the brine shrimp at 10 ppm.
- The genotypes Sunflower, Wabash, and Wells displayed the lowest acetogenin activity, killing less than 60% of the brine shrimp at 10 ppm.
- The remaining genotypes displayed intermediate acetogenin activity.

Objective

- The objective of this study was to assess the acetogenin activity of ripe fruit from 16 different pawpaw genotypes.

Materials and Methods

- Pawpaw fruit were collected from 16 pawpaw genotypes in September 2005. Pulp from at least 5 ripe fruit were extracted from skin and seeds, placed in zip-lock bags, and stored in a -15° C freezer.
 - Extraction of Acetogenins and Brine Shrimp Testing (BST). Acetogenins were obtained by extracting 9 grams of freshly thawed pawpaw pulp with 95% ethanol. Following quantification a 2.5 mg/ml solution was made using 95% ethanol, and 1, 2, 10, 20, 200, and 2000 µl were added to 2 dram vials to correspond to 0, 0.5, 1, 5, 10, 100, and 1000 parts per million (ppm). After the ethanol was evaporated twenty brine shrimp larvae, taken 48 h after initiation of hatching in artificial sea water, were added to each vial, and the final volume of each vial was adjusted to 5 ml using the artificial sea water. After 24 h, survivors were counted. Each sample had at least three replications.

Results

Table 1. Brine shrimp test (BST) for evaluating brine shrimp survival in extracts from fresh pawpaw pulp at a concentration of 10 ppm.

Genotype	% Mortality
Middletown	91.83 ± 8.52
Mitchell	88.55 ± 2.51
NC-1	94.17 ± 8.43
Potomac	76.67 ± 12.58
Sunflower	54.62 ± 22.17
Susquehanna	86.57 ± 10.42
Taylor	93.33 ± 7.64
Taytwo	92.27 ± 7.66
Wabash	51.72 ± 35.79
Wells	60.00 ± 5.00
Zimmerman	70.00 ± 10.00
2-10	96.67 ± 5.77
3-11	90.00 ± 10.00
10-35	83.50 ± 14.51
11-13	96.67 ± 5.77
K2-7	75.83 ± 17.85

Discussion

- Brine shrimp mortality varied with different pawpaw genotypes indicating varying acetogenin activity levels.
- Brine shrimp mortality varied between 52% and 96% at 10 ppm.
- Yields indicate ripe pulp can serve as a new large source of biomass for the extraction of acetogenin compounds.
- Further studies will be needed to determine additional high and low acetogenin activity genotypes.

Conclusion

- Pawpaw genotypes contain varying levels of acetogenin activity, indicating that some genotypes would be more suited for commercial production of acetogenins than others.

Literature Cited

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