Tracing the Migration of Pesticides through the Production of Southwestern Connecticut Honey

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Jose Manual Otero Award for Outstanding Research

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Abstract:

Continued use of pesticides and herbicides throughout both the agricultural and private sector has led to concern about the effects of these chemicals on common fruits and vegetables, and whether these often harmful chemicals are still present when the food is consumed. Most research has focused on the growth and retail of typical agricultural products, however little work has been done to identify the presence of residual pesticides in the honey of Apis mellifera (the typical honey bee). This investigation seeks to verify the migration of five known pesticides, applied within a 30 mile radius within the Bartlett Arboretum (in Stamford, CT), to the honey product of a controlled bee-hive located within the Arboretum grounds. The five pesticides applied within the Arboretum are Dipel, Neem Oil, Insecticidal Soap, Imidacloprid, and Pyrethrins. Initial experiments based on ultrasonic-solvent extraction of honey, and the analysis of the organic layer against known samples of the five pesticides, with Attenuated Total Reflectance (ATR) Fourier Transform Spectroscopy (FTIR), proved to be non-specific and lacked sufficient sensitivity since all pesticides, and the neat honey samples exhibited an asymmetric C-O stretch at 1058 $cm^{-1.}$ High Pressure Liquid Chromatography based analysis, with UV detection at 255 nm, indicates that components of Pyrethrins and Imidacloprid are not detectable in the organic honey extract. This same analysis points out, however, that components of BioNeem Oil are present in the final product of the Arboretum 2007 Fall Honey.

Introduction:

The use of pesticides is an essential aspect of modern agriculture. These pesticides can be used to control insects, weed growth, plant diseases, along with worm and rodent infestations. Fruit and flower growers in the state of Connecticut use pesticides primarily to control insect infestation. Although pesticides are very effective in controlling the population growth of unwanted insects, they are usually non-toxic to honeybees. In part, the lack of toxicity to honeybees can be attributed to the timing of the pesticide application. Farmers methodically spray their crops at night, cover or even move beehives to minimize the direct contact with honeybee populations until the pesticides have become less hazardous. The pesticides remains on the plant surfaces, and although the pesticide does not kill the honeybee, those that feed on the nectar and pollen from these treated flowers can easily carry the residues into the hive and introduce the pesticide into the honey that is produced.

Establishing a working relationship with the Bartlett Arboretum in Stamford, CT, a wildlife preserve possessing 30 acres of gardens and woodland, I was fortunate enough to have the opportunity to monitor the production of honey from a specific beehive located on the arboretum grounds.

Figure 1. The location of the beehives on the Arboretum Grounds

The honeybees within the centrally located controlled hive are known to feed off of flowers in a five-mile radius surrounding the arboretum according to Mr. James Kaechel (the Director of Education, and bee specialist) and Mr. Andrew Coté (the Stamford Arboretum Beekeeper). The pesticides used within this five mile radius are limited to Dipel, Neem Oil, Insecticidal Soap, Imidacloprid, and Pyrethrins, the most hazardous of the pesticides used on the grounds. With the Assistance of Mr. James Kaechel and Mr. Andrew Coté I collected samples of fall honey harvest from the controlled beehive and located samples of the five commercial-grade pesticides, used in the neighboring locations where the bees feed.

Purpose:

The investigation of the subsequent transmission/migration of applied pesticides into the final honey product.

Hypothesis:

In a controlled environment, where the applied pesticides in a five-mile radius are known, and the bee colony feeding is limited to these same locations, trace levels of pesticides will be found in the final honey product.

Materials:

- Pesticides obtained from Bartlett Arboretum including:
    - Dipel
    - Neem Oil
    - Insecticidal Soap
- Imidacloprid Standard (Riedel-de Haën Item 37894)
- Pyrethrins extracted from chrysanthemum daisies
- Raw Honey obtained from Bartlett Arboretum
- Pipettes
- Perkin Elmer Spectrum 1 FTIR With A Diamond ATR Sampling Accessory
- Water Module 1 HPLC System with Acetonitrile & Water as the Mobile Phase

Methods:

The initial procedure involved the characterization of the pesticides used at the Bartlett Arboretum using Diamond Attenuated Total Reflectance (ATR) Fourier Transform Infrared (FTIR) Spectroscopy at the Greenwich High School Labs.

Figure 2. *ATR FTIR spectrometer*

This technology helps to understand the interactions between the different atoms within a molecule and produces a spectral profile of the specimens being examined collecting high

quality data within a minute or less. The procedure began by sampling the five pesticides with the Diamond ATR FTIR by placing liquids on the metal plate and isolating solids with the high pressure clamp, providing close contact between the sample and the ATR crystal.

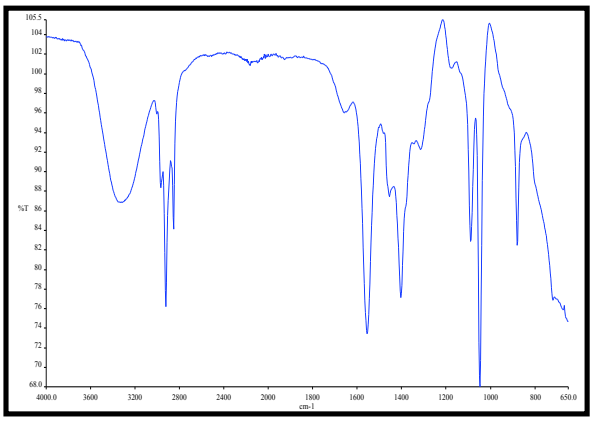


Figure 3. *ATR FTIR Spectrum Insect Killing Soap*

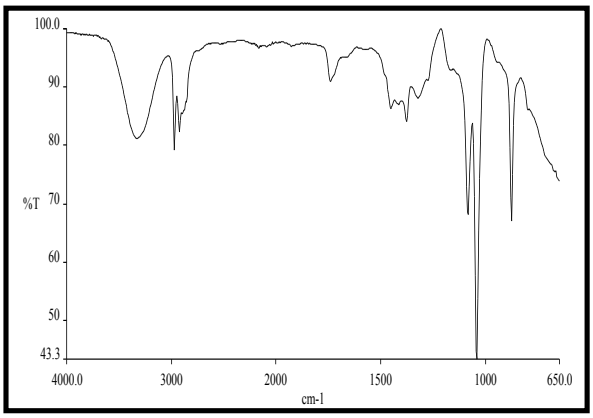


Figure 4. *ATR FTIR Spectrum of BiNeem Oil*

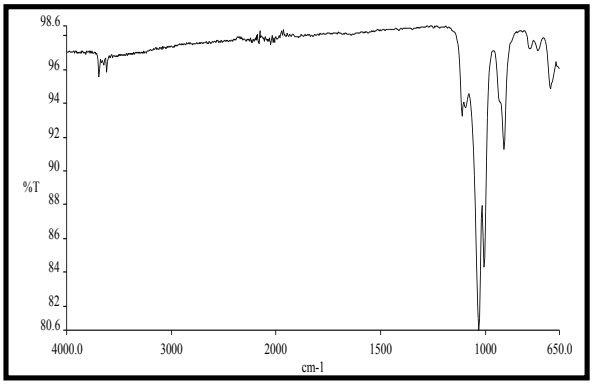


Figure 5. *ATR FTIR Spectrum of Garden Dust Dipel*

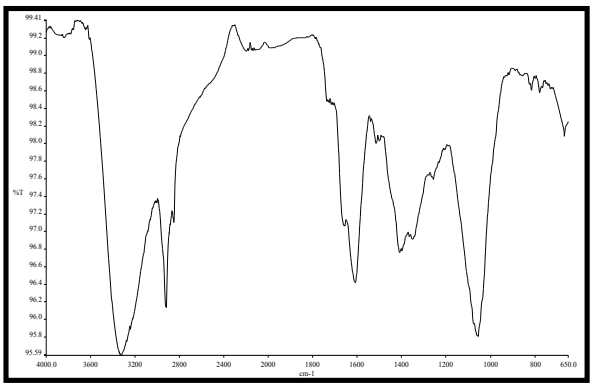


Figure 6. *ATR FTIR Spectrum of Pyretherin*

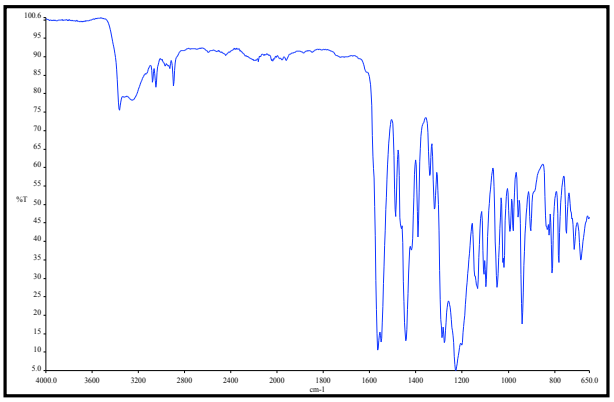


Figure 7. *ATR FTIR Spectrum of Imidacloprid Standard*

The ATR FTIR spectra of the five pesticides all exhibited a peak at 1058 cm^{-1}, which is attributed to an asymmetric C-O stretch within a molecule contained in the pesticide.

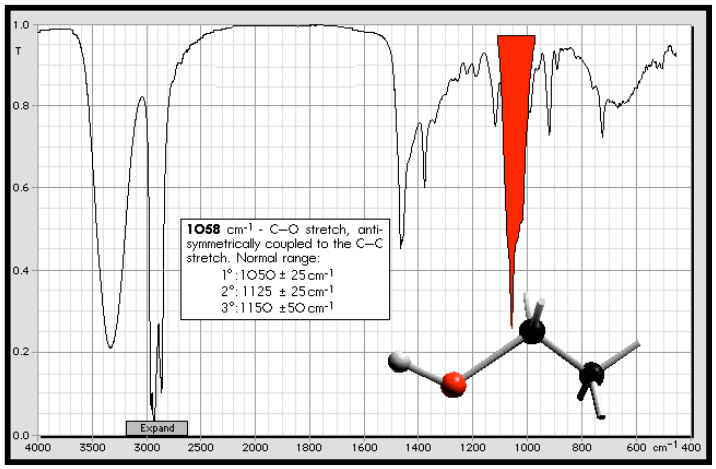


Figure 8. *All Pesticides Share a C-O stretch at 1058 cm^{-1}*

Using the Ultrasonic Extraction of Pesticide from honey, a commercial honey product was spiked with Dipel pesticide to check the detection of the pesticides in honey. 1.0 grams of honey were dissolved in 1.25 grams water mixed with 2.5 grams of Heptane. An ultrasonic mixer was used to combine the two layers for 60 minutes at room temperature. This distinguished between the water soluble, hydrophilic components that stayed in the aqueous layer and the non-polar, hydropholic components that migrated into the Heptane layer.

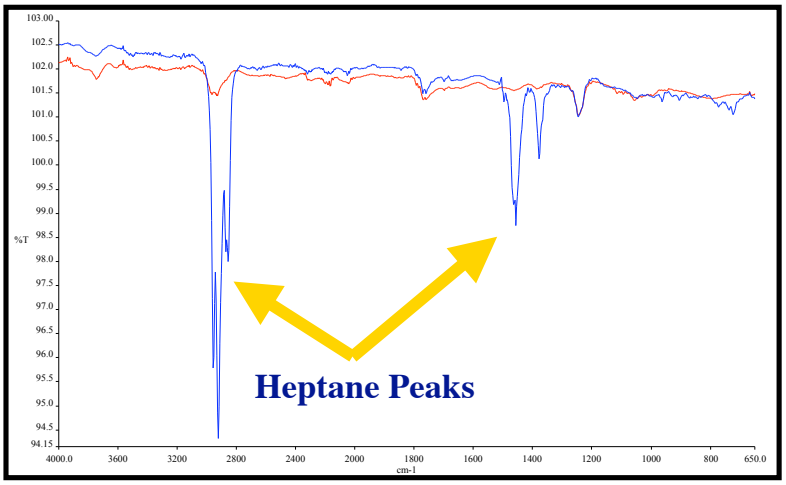


Figure 9. *The arrows indicate the presence of the heptane peaks*

Taking an ATR FTIR Spectra of Honey Extract (Wet Heptane Layer) and Dry Film after evaporation, demonstrated after the evaporation of the Heptane layer pesticides were undetectable via ATR FTIR. Therefore it was concluded that the FTIR methodology

lacked the sensitivity required to distinguish between the components in the scans by looking at the Heptane layer.

Analyzing a sample of "neat" Arboretum Honey, the FTIR indicated that the raw honey also produced a C-O asymmetric stretch at 1058 cm^{-1}.

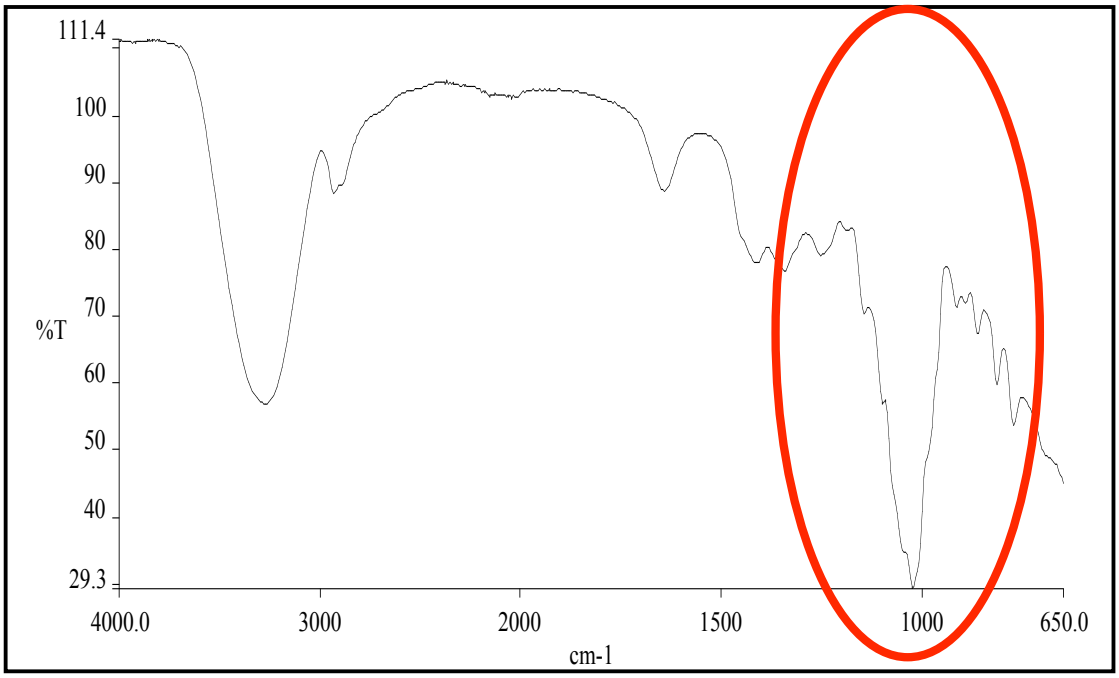


Figure 10. *A "Neat" Sample of Arboretum Honey*

This peak coincided with a peak in all other FTIR of the pesticides used on/around the Arboretum and concluded that the FTIR technique was non-selective. This specified that it was not possible to identify any of the pesticides independent of one another.

Deciding to utilize a new approach, a more careful investigation of the presence of Pyretherins was chosen because they are the most hazardous of the five pesticides. Pyretherin is a pesticide commonly found in low quantities in foggers and garden sprays. Use of Pyretherins from one of these foggers or sprays, would introduce many other

unidentifiable compounds that would complicate the analysis. Therefore, to create pure Pyretherin, the pesticide was extracted directly from Chyrsanthemums (i.e. the pyrethrum daisy) flower heads using isopropyl alcohol (91%). Leaving the solution overnight, the flower heads excreted the natural pesticide.

Previous literature by Kasaj, D., et al suggests that Pyrethrins can be detected via HPLC (High Pressure Liquid Chromatography). All HPLC work was performed in the labs at Greenwich High School.

My new approach involves the examination of the Heptane-layer of the Arboretum Honey extract, and the comparison of it to the HPLC of the chrysanthemum Pyrethrin standard. A new possible method was devised to extract the Pyrethrins from honey by taking 1.5 g of Chrysanthemum Heads in 20 ml IPA. 10 ml of Pyretherin/IPA Extract with 10 ml was mixed with 1:1 Methanol:Water and loaded onto a C_{18} Prep Column.

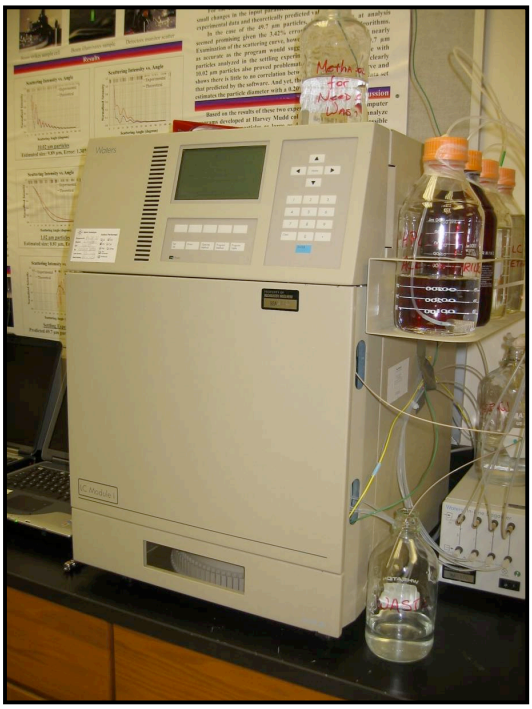


Figure 11. *HPLC Chromatography at GHS Labs*

The function of the column is to separate the water soluble substances from the organic components that are hydrophobic. After the Pyrethrins are passed onto a C_{18} cartridge, a 5 ml MeOH/Water wash is used to remove any remaining water soluble components and 10 ml of Heptane is used to remove the Pyrethrins from the C_{18} Prep Column.

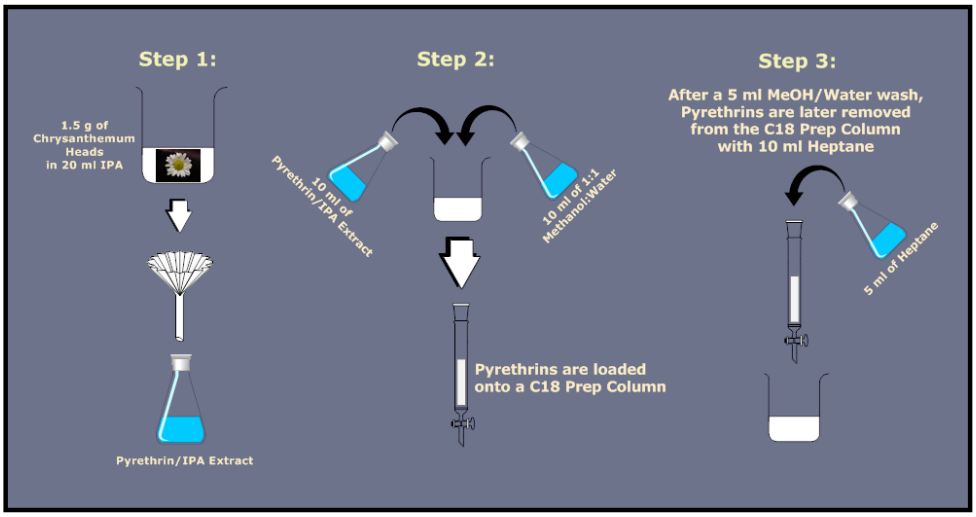


Figure 12. *Pyretherin Extraction & Purification from Chyrsanthemum*

The UV/Vis Spectrum of the Pyrethrin (in Heptane) showed significant absorbance in the UV region. 255 nm was selected as the wavelength of detection for HPLC analysis.

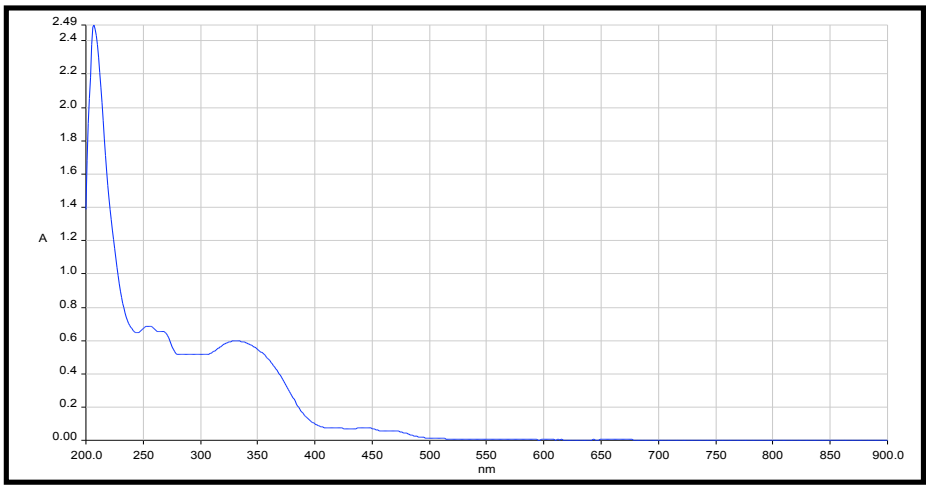


Figure 13. *UV/Vis Detection of Pyrethrins*

The Gradient HPLC method outlined by Kasaj provided poor separation for the Pyrethrins extracted from the chrysanthemum daisy. A modification of this gradient HPLC method outlined by Kasaj, et al, provided the best separation of the Pyretherin isomers:

Table 1: Gradient mobile phase parameters

Time (min)	% Acetonitrile	% Water
0	58	42
5	58	42
35	75	25
46	100	0
47 - 60	58	42

Additional HPLC parameters include:

- Column Type: Agilent Zorbax RX-C18, 250 x 4.6 mm
- Flow Rate: 1 ml/min
- Temperature: 23˚
- Wavelength: 255 nm

The HPLC chromatogram of the Pyrethrin Heptane extract and the Heptane (solvent) blank uncovered the presence of a Pyretherin II peak, a solvent peak, and a Pyretherin I peak. The chromatogram of the Heptane blank was collected to verify any contributions of the solvent to the Pyrethrum chromatogram. The Heptane blank contains a peak at a retention time of 9.2 minutes, so this peak can be assigned as a blank component in the Pyrethrin chromatogram as well. Using previous chromatography by Kasaj D., et al. Pyrethrin II and Pyretherin I were assigned retention times of 8.4 mm and 9.65 minutes respectively.

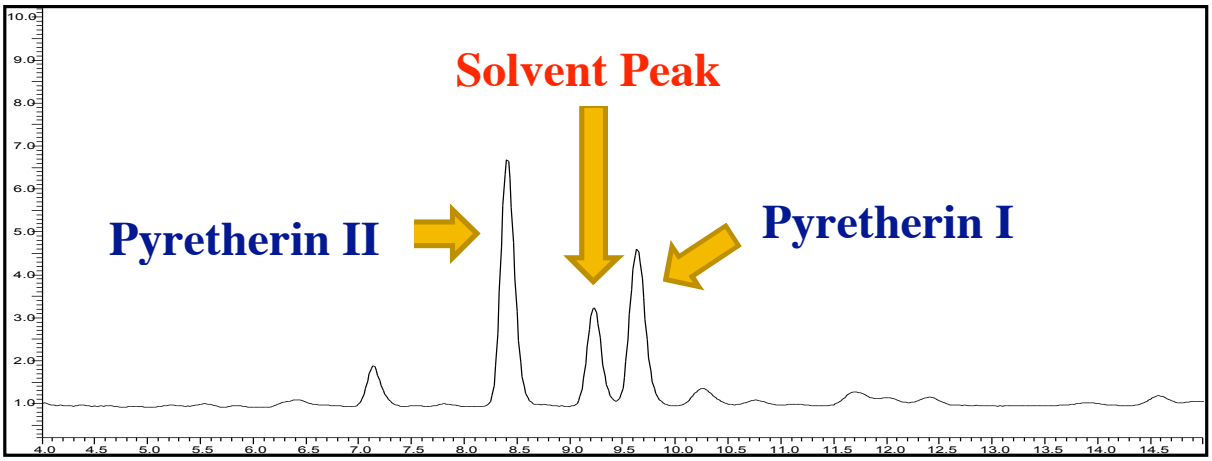


Figure 15. *Chromatogram of Pyretherin Heptane Extract*

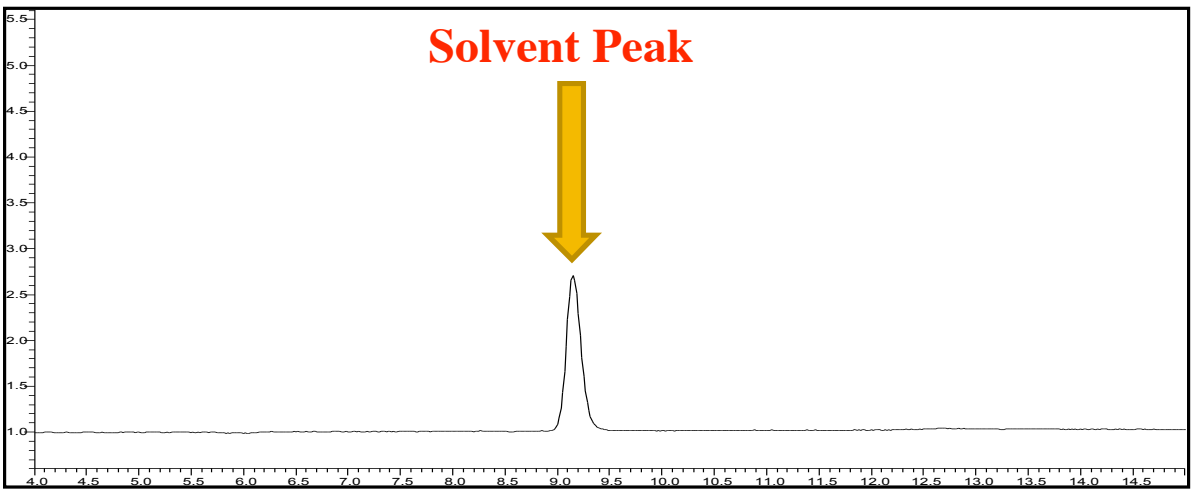


Figure 16.Chromatogram of Heptane (solvent) Blank

A similar method to extract possible Pyrethrins from honey was devised. 5 grams of a honey sample were mixed with 10 ml of 1:1 Methanol:Water. The organic components Methanol/Water mix is loaded onto a C18 prep column. Then after a 5 ml MeOH/Water wash, the organic components are later removed from the C_{18} Prep Column with 5 ml of Heptane.

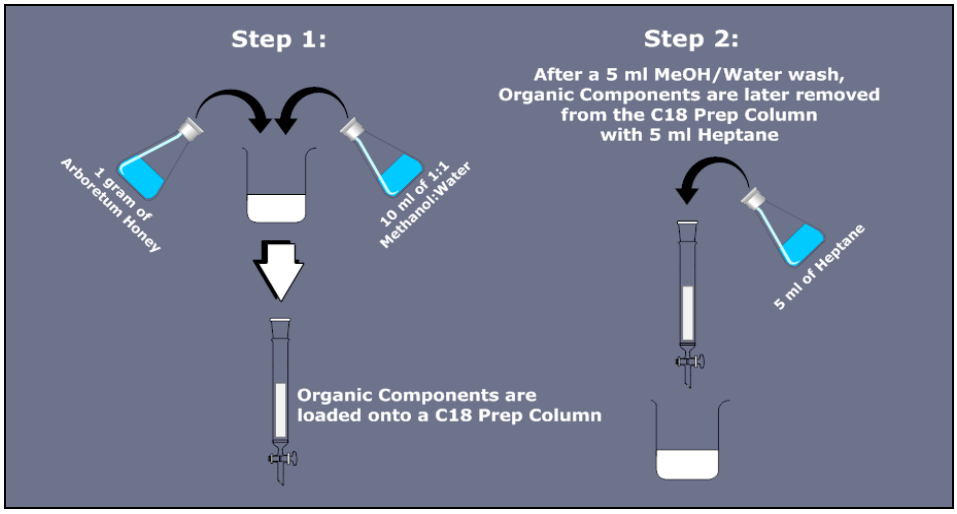
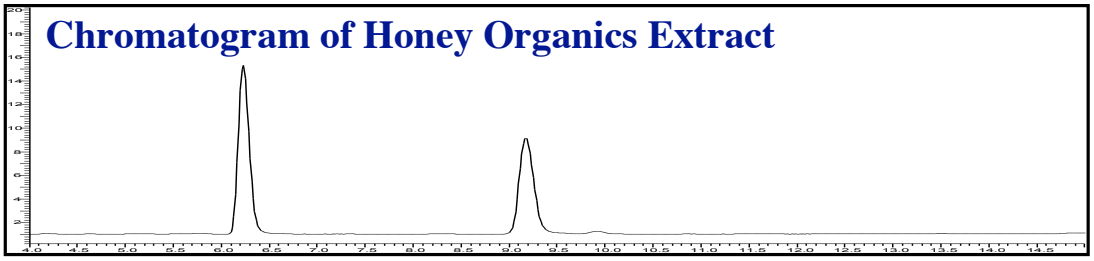
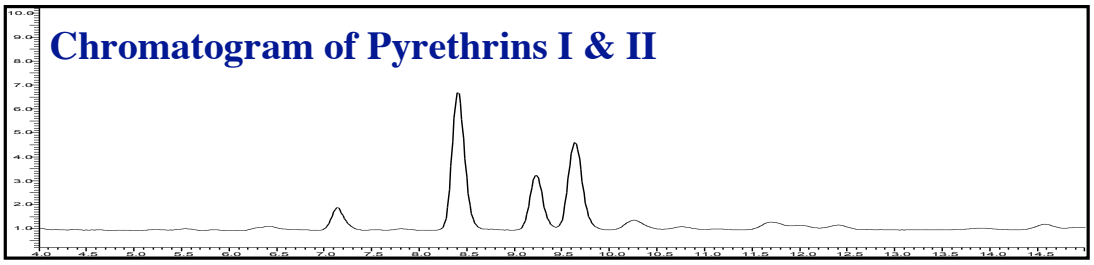
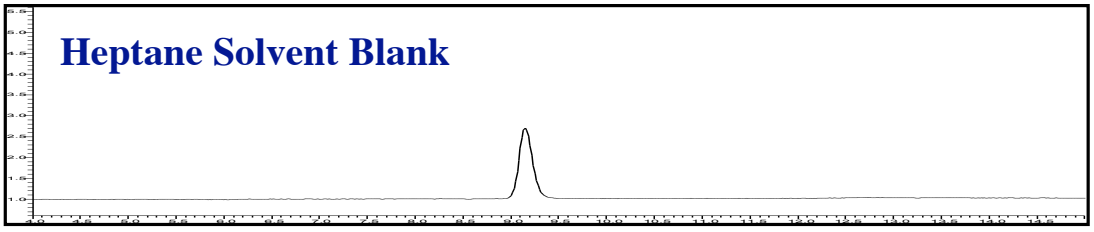


Figure 17. *Extraction of Organic Components from Honey*

The HPLC chromatogram of the Honey Organics Extract were taken in Heptane was collected and compared to the Chromatograms of Pyretherins I & II, and the Heptane (solvent) blank.







The comparison of the chromatograms revealed the presence of a new peak in the honey organics extract that did not correlate with the other chromatograms.

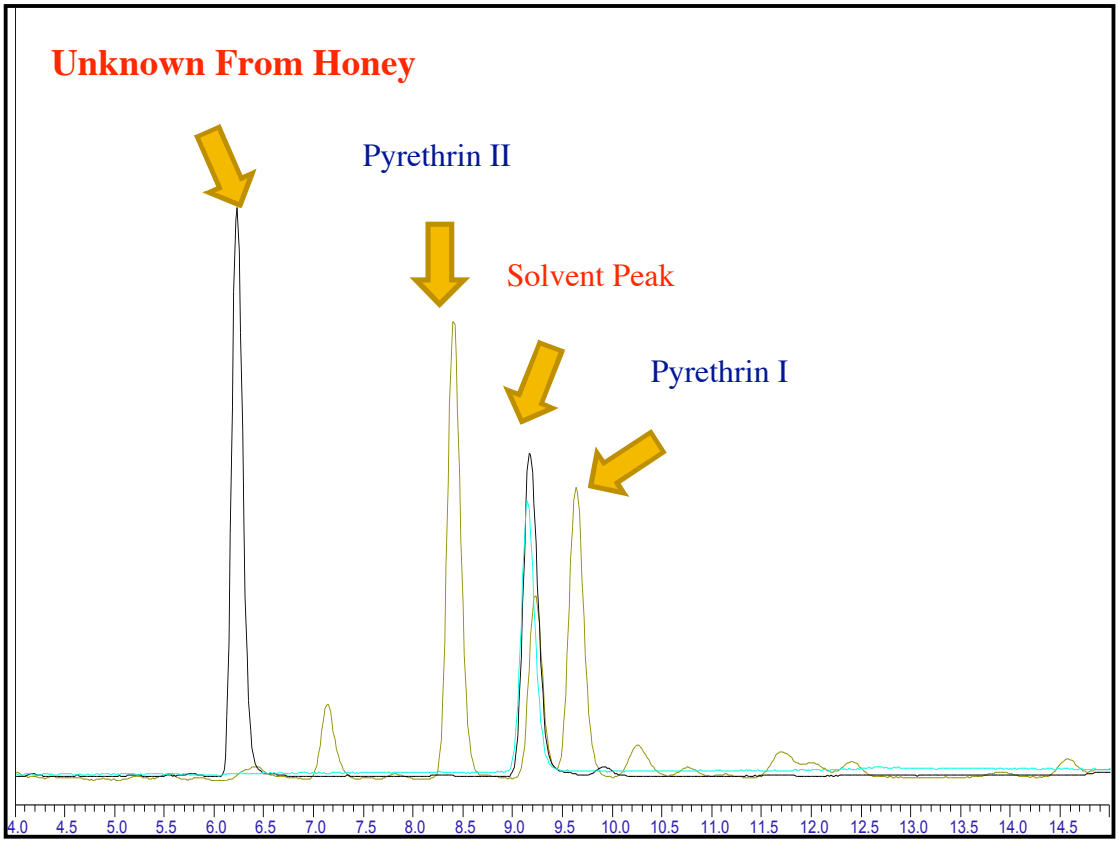


Figure 19. *Overlay of Chromatograms of Pyretherin with Honey Extract & Solvent Blank*

Of the five pesticides that were used at the Arboretum, Dipel and Insecticidal Soap are both water soluble and could not be detected with our new C_{18} Heptane Cartridge/Solvent Extraction method. Imidacloprid and Bio Neem Oil, however, both cointain pesticides that are hydrophobic and would elute with Heptane, so that I could compare their chromatography with that of the Arboretum honey organics extract. Imidacloprid was used to replaced its counterpart, Diazanon, a more powerful and effective pesticide used on field crops and around the home. With the recent ban of Diazinon, the use of Imidacloprid rose significantly amongst crop growers. It has been

theorized that perhaps this pesticide may be responsible for instigating Colony Collapse Disorder (or CCD), a little-understood phenomenon in which worker bees from a beehive abruptly disappear. CCD was originally termed in 2006, when nearly a quarter of U.S. honeybee colonies disappeared within a few months. The increased use of Imidacloprid coincides with the onset of CCD and many suggest that the pesticide may play a role in CCD. The presence of Imidacloprid in honey would define the pesticides pathway I the honey production, and shed light on its role in CCD. Imidacloprid is also harmful to humans by ingestion or inhalation.

A UV/Vis scan of the Riedel-de Haën Imidacloprid standard (in Heptane) shows an absorbance at 255 nm so Imidacloprid would be detectable with our current HPLC method.

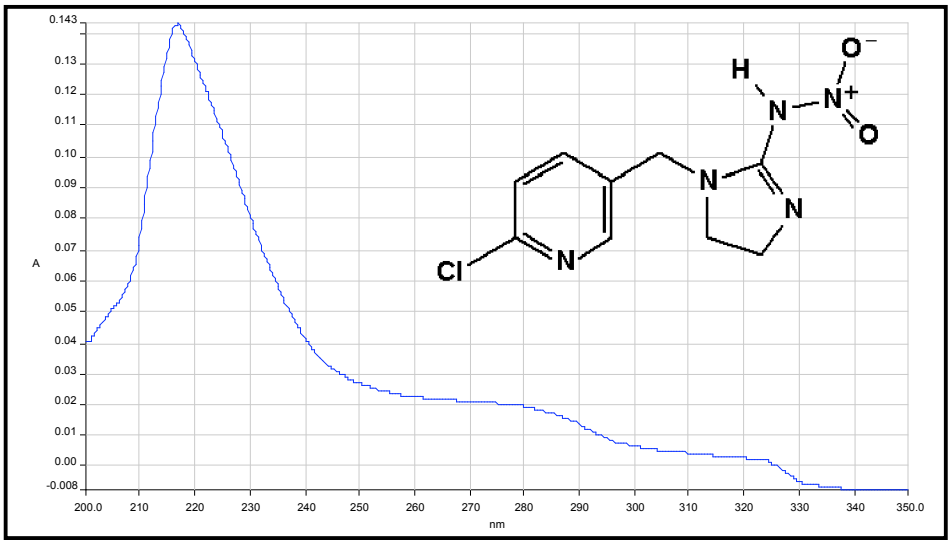


Figure 20. *UV/Vis Spectrum of Imidacloprid*

A chromatogram of the Imidacloprid was taken (in Heptane) revealed that the Imidacloprid eluted at a retention time of 52 minutes using the same HPLC method parameters.

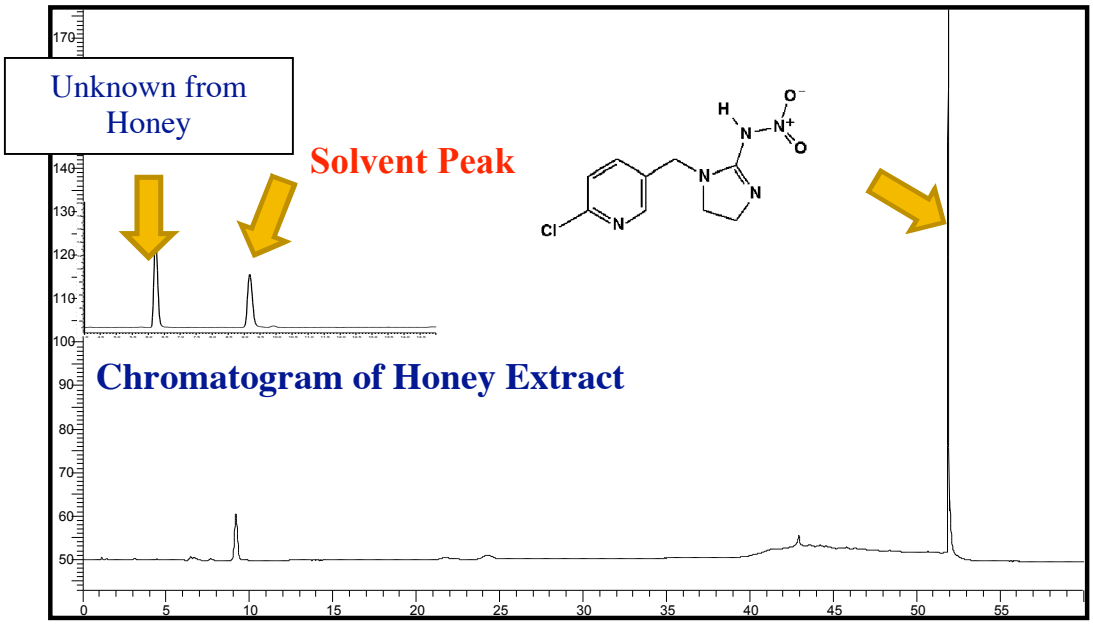


Figure 21. *Chromatogram of Imidiclorpid with Overlay of Honey Extract*

An overlay of the honey organics extract chromatogram (see figure above) with that of the Imidacloprid reveals that the Arboretum honey does not contain Imidacloprid.

A similar method to extract organic components from commercial BioNeem Oil pesticide was devised.

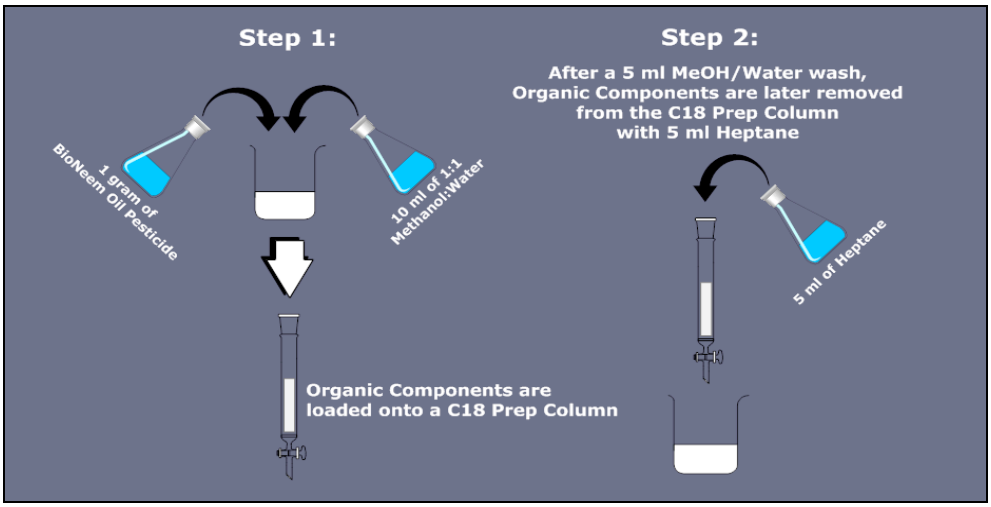


Figure 21. *Extraction of Organic Components from Bio Neem Oil*

Bio Neem oil contains the pesticide Azadirachtin and also exhibits absorbance in the UV region at 255 nm.

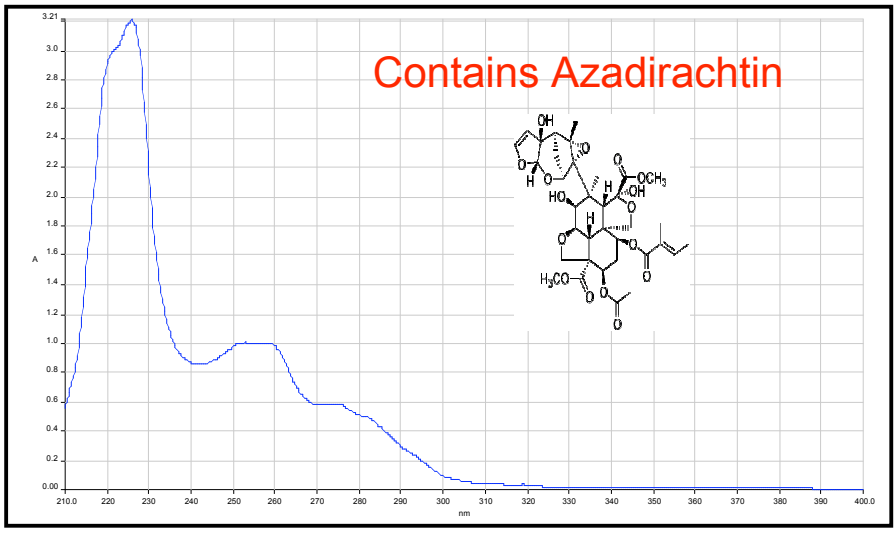


Figure 22. *UV/Vis Spectrum of BioNeem Oil – Heptane Extract*

As such, the same HPLC methodology can be used to detect the organic components of BioNeem in Heptane, after separation from any hydrophilic components, using the same C-18 cartridge/Heptane Extraction technique. The chromatogram for the organic components of BioNeem oil in shown below:

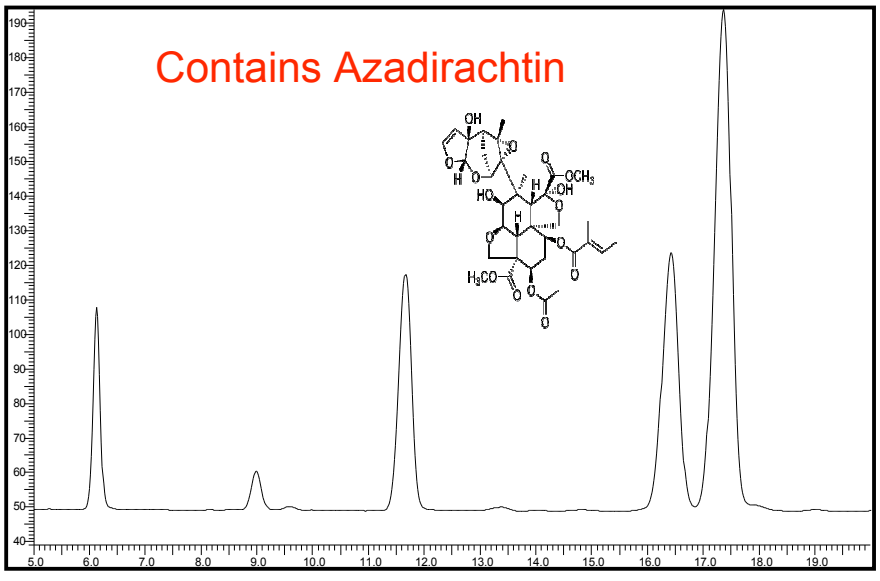


Figure 23. *BioNeem Oil Chromatography*

The Chromatogram of the BioNeem oil revealed the Heptane (blank) peak, as well as four other components with retention times of 6.25 min., 11.6 min., 16.4 min., and 17.4 minutes.

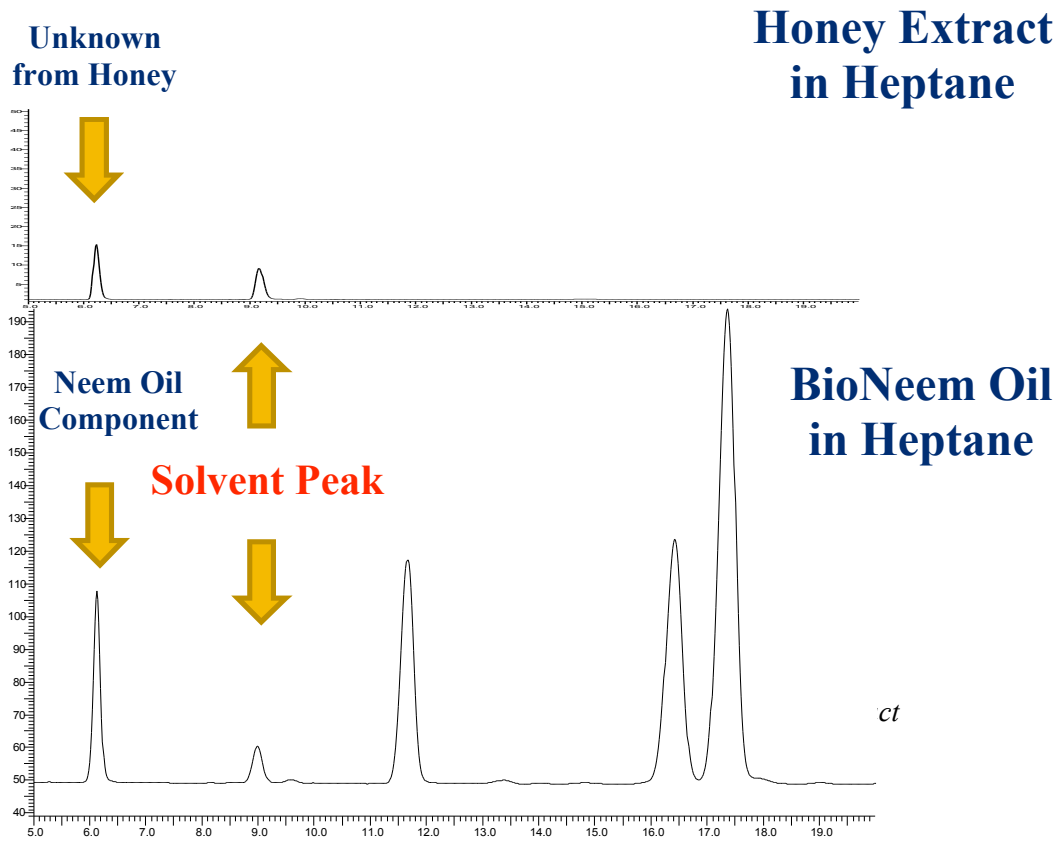


Figure 24. *Comparison of BioNeem Oil Chromatogram and Honey Extract Chromatogram*

The peak at a retention time of 6.25 min. coincides with the unknown component in the honey extract indicating that the Arboretum Honey final product does contain a component of Commercial BioNeem Oil pesticide.

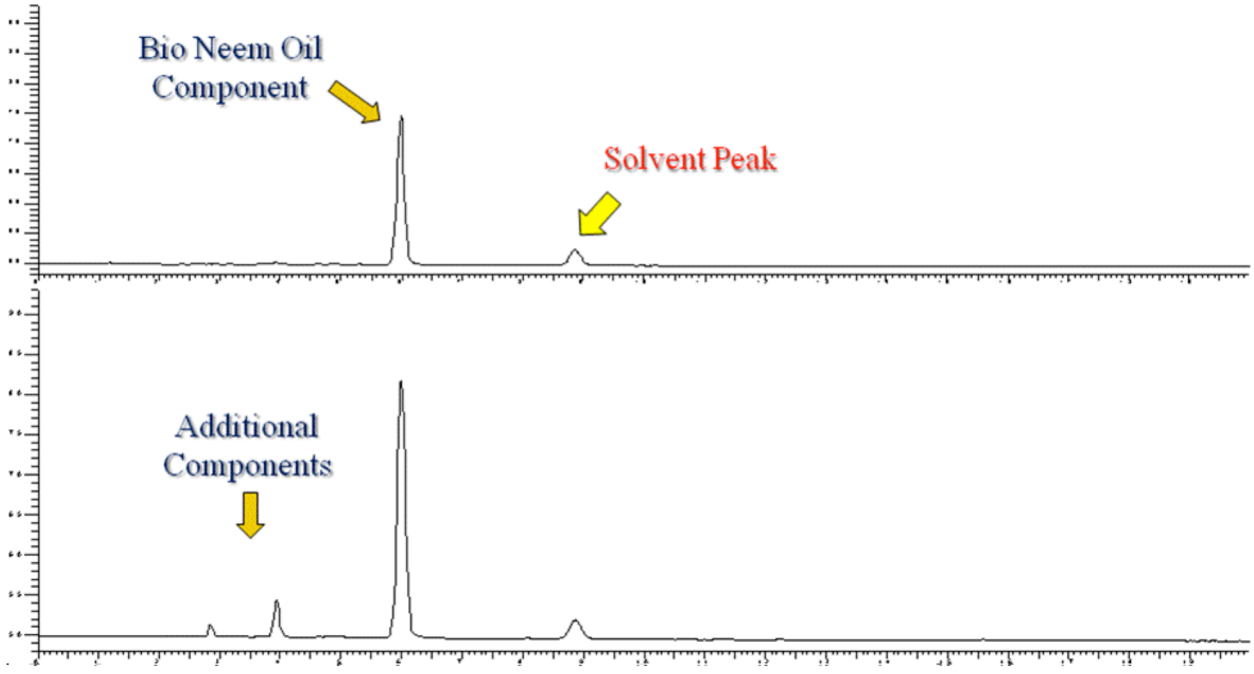


Figure 25. *Commercial Honey Analysis*

Preliminary analysis of commercial honey samples also reveal the presence of this same Bio Neem Oil component at 6.25 minutes, as well as additional unknown components with retention times of 2.85 and 3.95 minutes.

Conclusions:

Results from High Pressure Liquid Chromatography analysis of Bartlett Arboretum Honey support my original hypothesis; a component of Bio Neem Oil is present in the honey final product of the Bartlett Arboretum 2007 fall harvest. HPLC also uncovered that Pyretherins and Imidacloprid pesticides are not detectable in the same honey final product. The absence of the Imidacloprd from the Honey could support the linkage of the pesticide to CCD, where worker bees are killed before returning to the hive. The limited analysis of Commercial Honey Products of unknown geographical

origin also reveals the presence of the Bio Neem Oil component, as well as additional organic compounds.

Future Work:

Additional research will include the continuation of the HPLC analysis of commercially available honey samples to screen for the Bio Neem component with a 6 min. retention time. A hyphenated Liquid Chromatography technique, such as LC-MS, will be used to identify this organic component of Bio Neem Oil. Finally, the HPLC Analysis will be extended to the water soluble pesticides sprayed at the Arboretum (Dipel & Insect Killing Soap).

Acknowledgements:

I would like to thank Mr. James Keachel, and Mr. Andrew Cote, at of the Bartlett Arboretum, in Stamford, CT, for their assistance in obtaining Fall Harvest Honey samples, and for providing the names/samples of pesticides used within the Bartlett Arboretum. I would also like to thank my mentor, Mr. Andrew Bramante, at Greenwich High School, for his guidance and support during my research and The Science Education Center, of Fairfield County. CT, for their financial assistance.

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