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# Bio-oil from the pyrolysis of canola, *Brassica napus*, and mustard, *B. carinata* and *B. juncea*, straw: the potential for insecticide development

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# Introduction

- Project:
  agricultural crop residue → bio-fuel, chemicals

- This presentation:
  → bio-oils with pesticidal activity

- Alternative to chemical insecticides:
  - Reduce use of fossil fuels:
    - Insecticide fraction extracted
    - Remainder of bio-oil: cheap fuel
  - Prevent development of insect resistance

# Introduction

- In Canada:
  - > 200,000 ha of mustard
  - Straw: under-used agricultural residue
  - Mustard seed residue has been applied as a soil amendment that can suppress pathogens and insect pests

- Great potential for:
  - Conversion to liquid bio-oil
  - Pest control application

# Objectives

1) Screen bio-oils of canola and mustard straw for insecticidal activity

2) Identify active compound(s) in bio-oil fractions

# Methods

- Fast pyrolysis in fluidized bed pilot plant:

  - 300 and 500 °C
  - 2 s vapor residence time

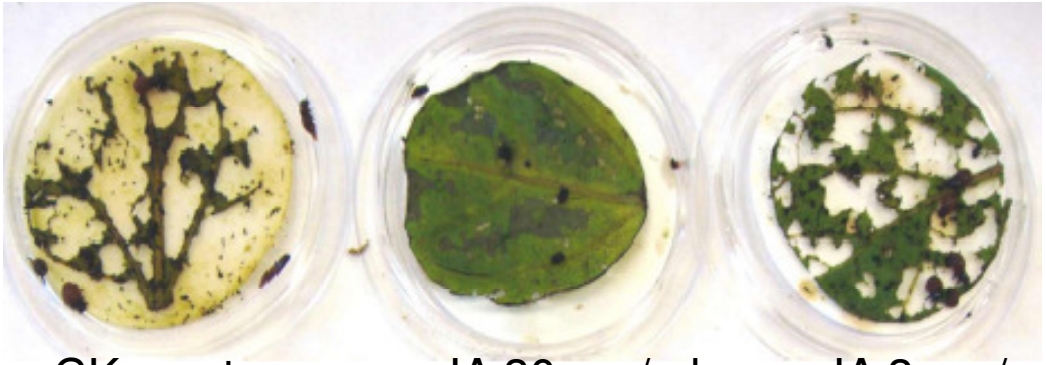
- Straw from:
  - canola *Brassica napus*
  - mustard *Brassica carinata*
  - mustard *Brassica juncea*

→ bio-oils:

- aqueous phase: *B. napus* (BNA), *B. carinata* (CA), *B. juncea* (JA)
- organic phase: *B. napus* (BNO), *B. carinata* (CO), *B. juncea* (JO)

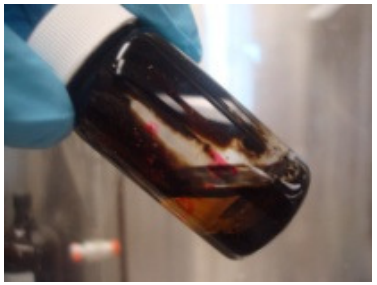
# Methods

- Tested for insecticidal activity using the Colorado potato beetle *Leptinotarsa decemlineata* (CPB) potato leaf disc bioassay

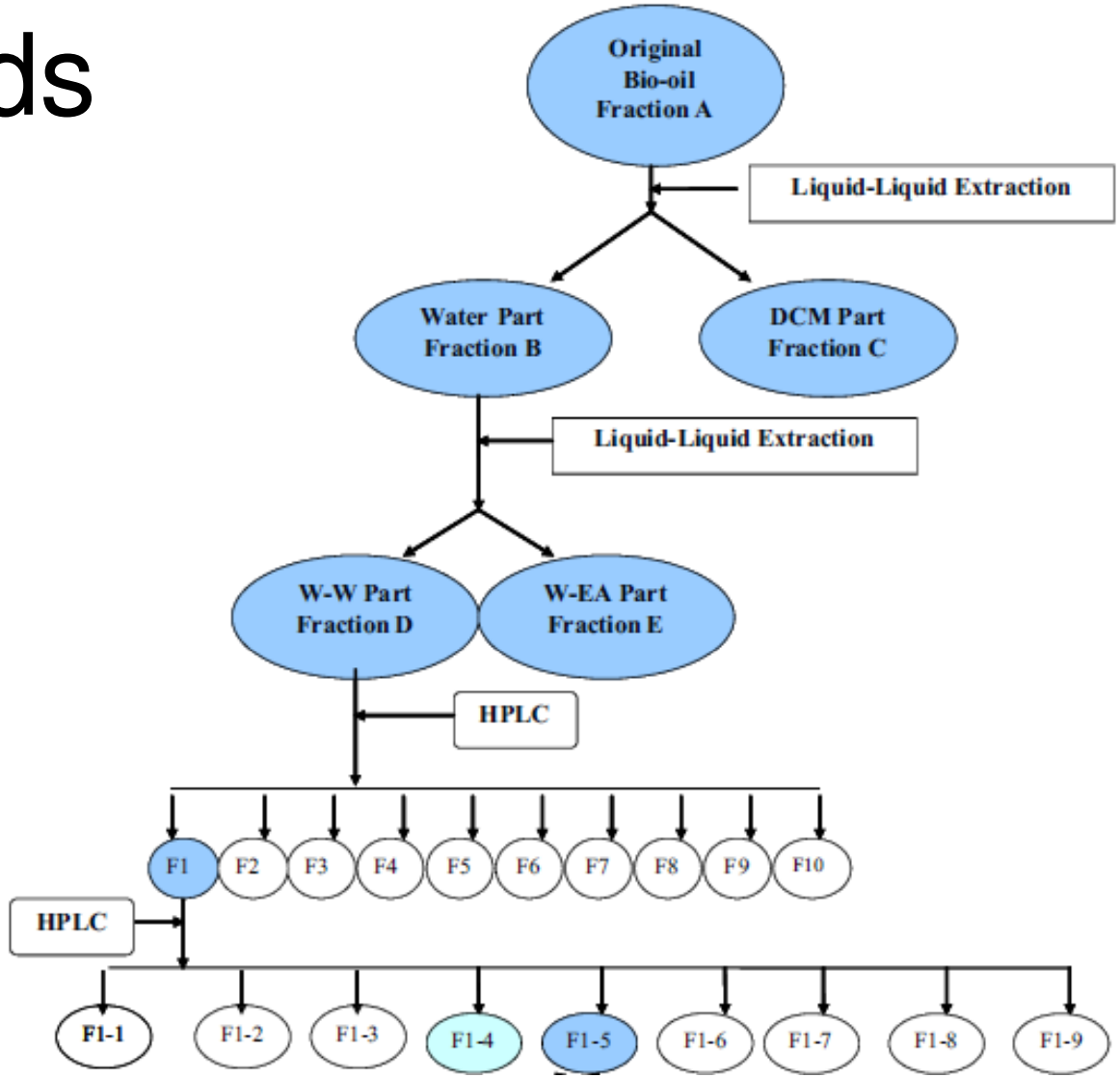


CK - water JA 30 mg/ml JA 3 mg/ml

- Raw bio-oil and separated solutions tested at 3 and 30 mg/ml



# Methods




- All fractions redissolved in water and acetone to an equivalent 30 mg/l concentration, for insecticide assays

# Methods

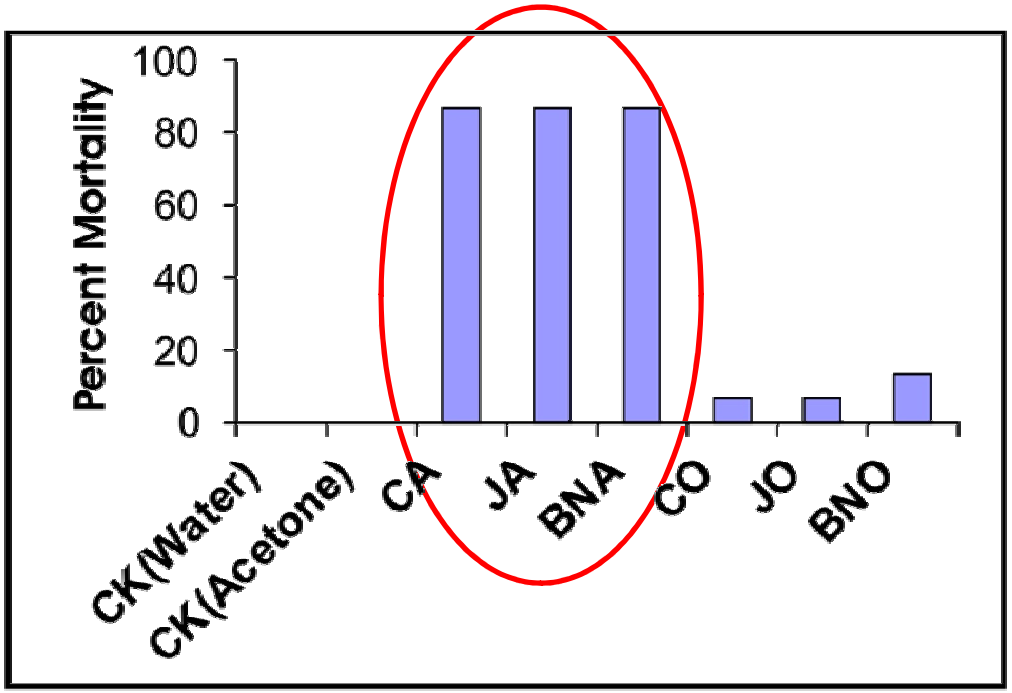
- HPLC fractionation method:

- Agilent 1200 Series HPLC
- Waters Symmetry C18 column (5µm, 4.6x250mm) for analysis
- Waters Symmetry semi-preparative C18 column (7µm, 7.8x300mm) for fraction collection

- HPLC analyses of known Brassicaceae compounds :

- Sinigrin and AITC from seed, straw and bio-oils from *B. juncea* were analyzed by HPLC (Agilent 1200)

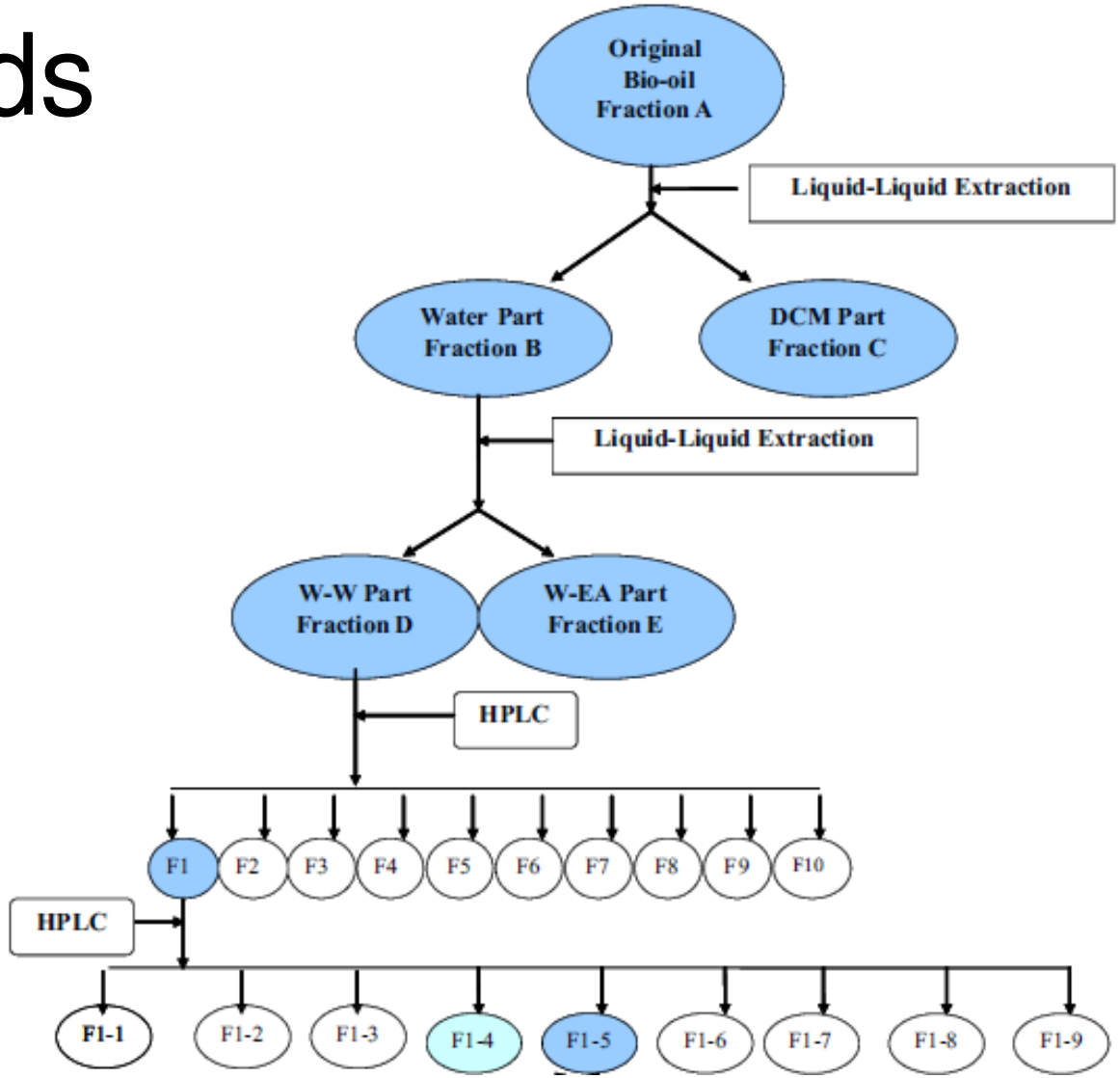
# Results




Tests with 30 mg/l against 1st instar CPB – 48 h % mortality

- All three *Brassica* aqueous phases (CA, JA, BNA) active
- Mustard bio-oils had higher bio-activity with aqueous versus organic phase (CO, JO)
- 300 ℃ bio-oils were more active than 500 ℃ bio-oils

# Methods

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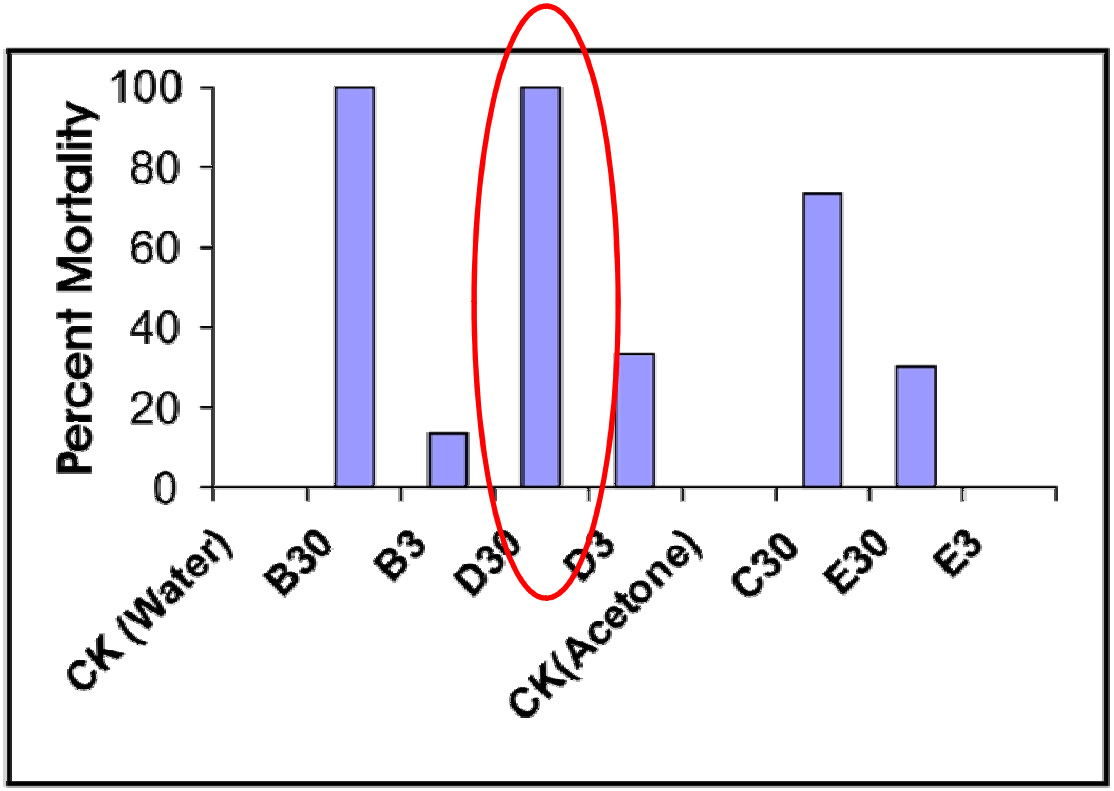
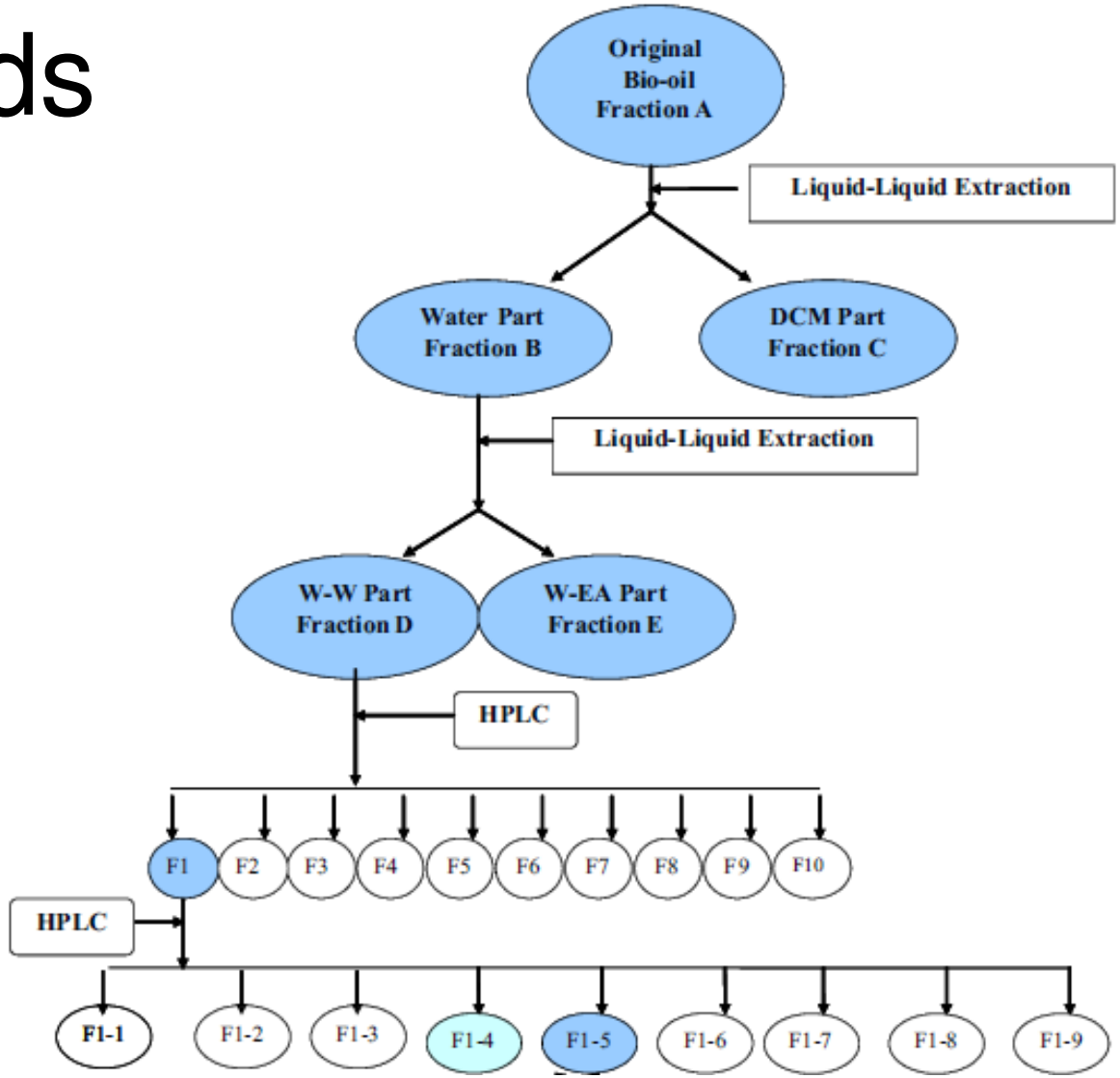



- Remaining studies with 300 ℃ oils
- Aqueous phase of *B. juncea* bio-oil chosen for further purification
- The 2nd aqueous separation retained the greatest activity in fraction D (30 mg/ml)

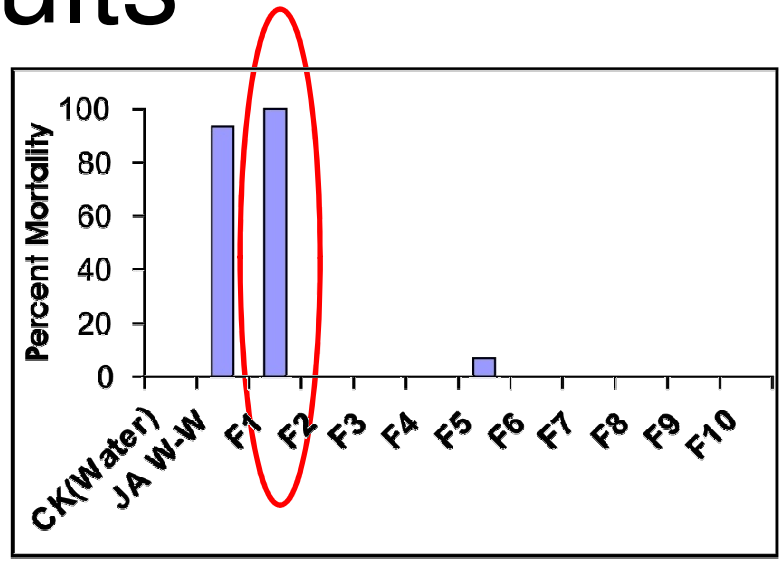
# Methods

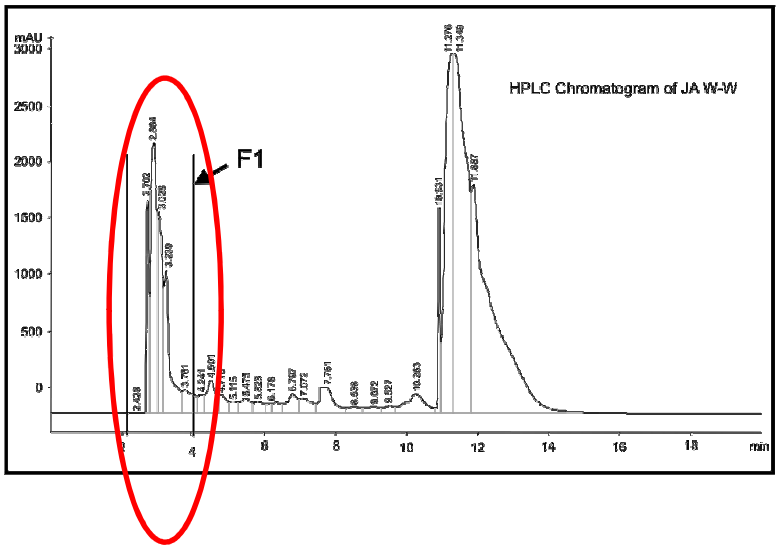



- All fractions redissolved in water and acetone to an equivalent 30 mg/l concentration, for insecticide assays

# Results

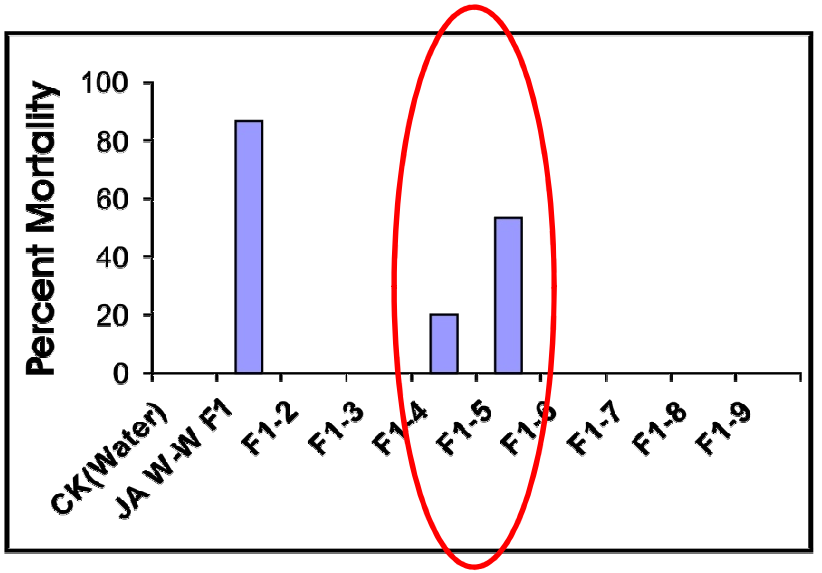
- Bioassay-guided fractionation by HPLC with semi-preparative C18 column
- 10 fractions of D30 were collected
- Fraction 1 was found to be the most active of ten sub-fractions collected and the most polar

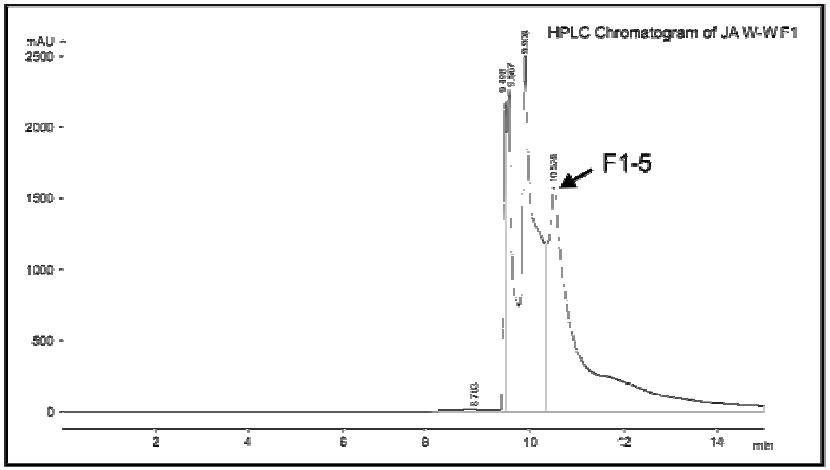


# Results

- Further HPLC purification of Fraction 1 produced 5 peaks and 9 sub-fractions were collected
- Insecticidal activity was found only in Factions 1-4 and 1-5
- Fraction 1-5 was the more active



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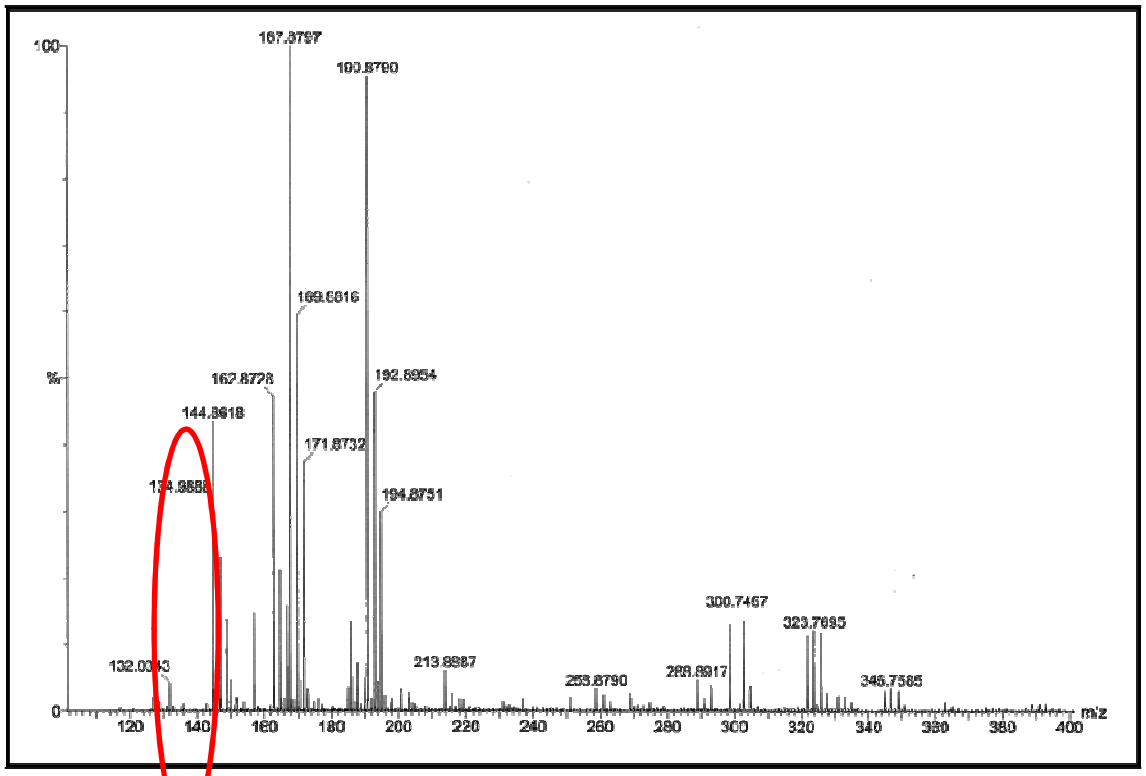


- LC-MS spectra: fraction 1-5 differed primarily in the amount of just one compound
- This probable active compound has a molecular mass of 134

# Conclusions

- Insecticidal activity in *B. juncea* bio-oils not associated with glucosinolate, Sinigrin, or isocyanate, AITC
- Active compound
  - molecular mass of 134
  - likely contains an amide group
- The presence of this compound in the other active fractions of bio-oils from mustard and canola needs to be verified
- Separation of a insecticidal compound could provide a “value-added” product from mustard straw

# On-going Projects

- Repeat pyrolysis with new mustard straw sample
- Liquid-liquid separation of bio-oil completed
- Clean-up of aqueous phase with Solid Phase Extraction (SPE) and acetonitrile rinse

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