

ABP - Training Syllabus: Development and application of plant-derived pesticides

Bio-prospecting

Discovery, development and commercialization of products from nature

Discovery process

1. Selection of plant species for evaluation

- By random selection
- Based on traditional knowledge
 - More probability of success
 - More likely to be less toxic (non toxic)

2. Collection and extraction of the selected plants

- Collection
 - Requirements: Liquid nitrogen, gunny bags; secateurs; GPS, camera; data sheet, plant press, protective gear, gumboots, cool box, cryogenic envelopes, labels and pen, plant taxonomist,
- Sample tracking and database management: GPS, camera; data sheet, computer, PDA
- Storage
 - Freezer: -20, -80, Liquid Nitrogen, cold room, fridge -4, store
- Plant processing
 - Drying under shade
 - Freeze drying
 - Size reduction: ball mill, grinder, motor and pestle.
 - Cold press
- Extraction Procedure
 - Hydro-distillation: Heating mantle, distilled water; Clevenger apparatus; measuring cylinder, funnel, stop watch, weighing balance lab coat, gloves, un-hydrous sodium sulphate, ethanol, acetone, amber bottles. Rotor evaporator to remove excess hexane after drying essential oil
 - Solvent extraction: for more polar plant extracts by extraction of plant material using solvents of varying polarities, including Hexane, acetone, ethyl acetate, dichloromethane, chloroform, methanol and water. The plant material will be sequentially extracted by immersion in the solvents, beginning with the least polar. The extracts will be dried by removal of the solvents under vacuum.

3. Testing of plant samples for effectiveness against bee pests and disease vectors

- Laboratory bioassays
 - **Effectiveness: Against varroa mites, nosema, foul brood and fungal bee diseases.**
 - Fumigant toxicity bioassay against varroa mites (*V. destructor*) and honey bees (*A. mellifera*) for effectiveness and safety
 - Disc and deep well bioassay for screening plant-derived products against foul brood disease in honeybees
 - Disc and deep well bioassay for screening plant extracts against fungal bee diseases
 - Screening of plant-derived products for control of nosema disease in honeybees
 - Testing for safety - Bees(toxicity), humans (clinical trials), environment (non-target, persistence)

4. Identifying active chemical constituents

- Analysis of the plant extracts and derived compounds will be undertaken by chromatographic, spectrometric and spectroscopic methods including the following: column, gas and high performance liquid chromatography (CC, GC, and HPLC, respectively), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR).
- Quality Control/standardization

5. Plant-based pesticides development steps

- Formulation
 - Different constituents combined in precise ratios; active ingredient, carrier, preservative, colour, fragrance, delivery system.
- Optimising effectiveness, safety
- Trials – semi-field, field - Methodology for field testing will depend on the type and form of the product, pest and disease
- Protecting intellectual property – patents etc.
- Registration of the products
- Production
- Commercialisation – sales and marketing