



## Production and Testing of Novel Formulations of Entomopathogenic Fungi

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### Background

- Invert emulsions are promising formulations for entomopathogenic fungi (EPF) when applied as biological control agents of insects.
- The water content of these formulations supports germination of EPF conidia during application and subsequent penetration of the insect cuticle.
- Water in this type of emulsion is homogenized with a plant-based oil at high speed. This process encases the water molecules in oil to decrease the evaporation rate compared with an unformulated conidial suspension.
- Invert emulsions have been used to apply strains of *Metarhizium anisopliae* and *Beauveria bassiana* against mites, whiteflies, weevils, borers and bark beetles.

### Objectives

- Prepare an invert emulsion (water-in-oil type) containing Australian strains of *Beauveria bassiana* and *Verticillium lecanii*.
- Test the efficacy of this formulation against mealworms, *Tenebrio molitor*.

### Emulsion Preparation

- Invert emulsions consist of an aqueous phase and an oil phase mixed at a ratio of 1:1.
- Water phase contains sterile de-ionized water, a water-soluble emulsifier and glycerin.
- Oil phase contains a plant-based oil and an oil-soluble emulsifier.
- Different combinations and quantities of these ingredients should be tested to select the most appropriate combination for a particular strain of EPF.
- Criteria for selecting the most appropriate ingredient(s) and/or combination(s) are stability and viscosity of the prepared emulsion.
- If the two phases separate over time, the oil phase will become clearly visible at the top of the emulsion (Fig. 1).
- After screening and combination tests, the most appropriate local ingredients used in preparation of the selected emulsion were:
  - Water phase (50 % w/w) = 45.00 % (w/w) sterile de-ionized water + 0.75 % (w/w) Dehymuls® LE + 4.25 % (w/w) glycerin.
  - Oil phase (50 % w/w) = 48.00 % (w/w) Canola oil + 2.00 % (w/w) Tween 20.
  - To produce 200.0 g of emulsion requires: 90.0 g sterile de-ionized water, 1.5 g Dehymuls® LE, 8.5 g glycerin, 96.0 g Canola oil and 4.0 g Tween 20.
- The water and oil phases are prepared separately before adding the water phase to the oil phase. Next the formulation is homogenised at 20,000 rpm for 90s.
- The EPF is incorporated into the water phase of the emulsion as a conidial suspension before the two phases are combined and homogenised (e.g. 22.5 % w/w conidial suspension added to 22.5 % w/w of sterile de-ionized water).

### Bioassays

- 3 fungal strains were tested against early instar mealworms (Fig. 2, n = 10 larvae per dish).
- Three treatments of each fungal strain were applied (1 mL of solution per dish):
  - unformulated fungus = EPF in a water suspension, ii) blank formulation = invert emulsion without EPF, iii) formulated fungus = EPF in the invert emulsion
- Between 6-8 concentrations were tested for each treatment plus an untreated control, i.e. concentration = zero (Table 1, n = 3 replicate dishes per concentration).

Table 1: Fungal treatments applied

Fungal species and strain	Concentrations applied (conidia/mL)
<i>Beauveria bassiana</i> strain BG1	8 x 10-fold dilutions starting from 1.0 x 10 <sup>8</sup>
<i>Beauveria bassiana</i> strain BF1	6 x 10-fold dilutions starting from 3.0 x 10 <sup>6</sup>
<i>Verticillium lecanii</i> strain VLB	7 x 10-fold dilutions starting from 3.0 x 10 <sup>7</sup>



Figure 2: Mealworms (n = 10) reared on wheat bran for Petri dish bioassays

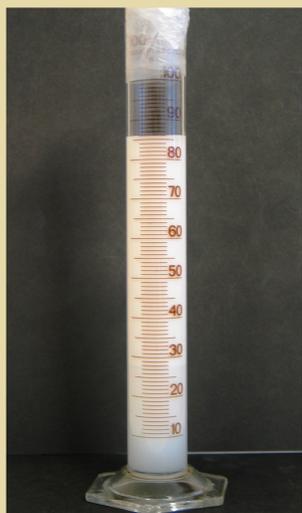


Figure 1: Prepared invert emulsion has a milky appearance with the two phases well-mixed.

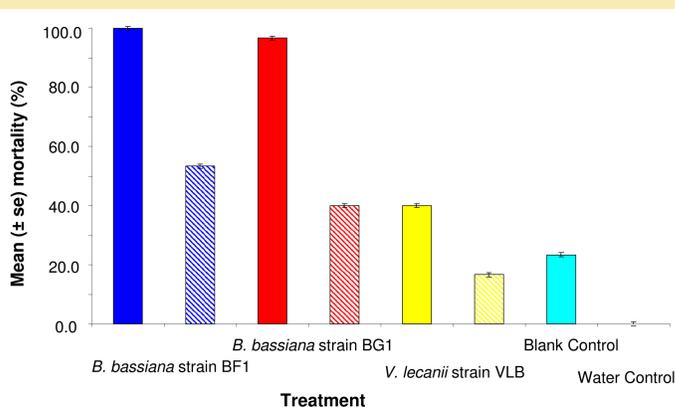


Figure 3: Mealworm mortality in response to highest concentrations of 3 EPF strains tested as formulated emulsion (solid bars), unformulated suspension (striped bars), blank and water controls. The 2 strains of *B. bassiana* were more effective than *Verticillium lecanii* although formulation improved efficacy of all 3 strains.

### Results



Figure 4: EPF-infected mealworms. Left to right, 1<sup>st</sup> 4 larvae show different levels of mycelial growth by *B. bassiana* (strain BG1) after being killed by the fungus (within 2-3 days after death under humid conditions), 5<sup>th</sup> larva killed by BG1 infection but does not yet show mycelial growth of the fungus (death within 5 days after fungus application), far right larva is healthy.

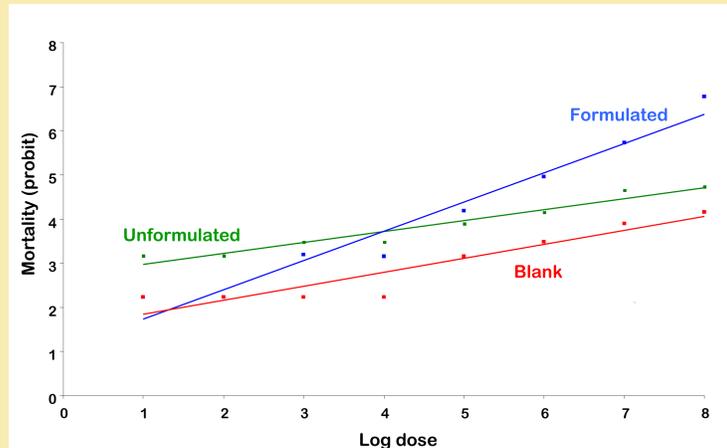


Figure 5: Dose response of mealworms treated with *B. bassiana* strain BG1 (formulated and unformulated forms and blank control). Mortality increased with concentration for all treatments but formulation increased mortality at higher concentrations compared with the other treatments.

#### Selected References

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