Insecticide Assay

* Final Well Volume = 200µl
* Plate reader settings: 30°C Number of cycles = 193, number of flashes per well and cycle = 22, cycle time = 300s, shaking options = double orbital, 200rpm, 272s, before each cycle.
* Create **4ml** 0.05 OD yeast culture stock and dilute down to final theoretical 0.01 OD.
* 1% final concentration for both solvents.
* P200 and P20 pipette.
* Fill plate perimeter with blanks to combat edge effect.
* Split plot randomisation with insecticides rotating clockwise each replicate.
* Repeat 8 times.
* 10 insecticides with 5 concentration values = 50 wells + 5 controls + 5 blanks = 60 wells.
* Use a heated Eppendorf block when creating stock solutions to combat solubility issue with Thiacloprid and Cypermethrin. Add the media, allow to heat up then add the stock solution. Hopefully will prevent precipitation.
* Slide master stocks down the side of the eppendorf. Aids dissolution.
* Create plates as usual. Before adding 0.05 yeast OD stock, put plate in incubator for 15 minutes to allow it to heat up to 30°C and mix. Whilst this is going on get the yeast out of the orbital shaker and dilute down to 0.05 OD. Keep a culture tube of media in the shaker too so also at 30°C.
* Store insecticides at 4°C as methanol evaporates at room temperature.
* Heat master solutions to 37°C before making working solutions.
* Use multichannel to add 0.05OD yeast.
* Fill perimeter with media.
* Make stock solutions **first.**
* When adding master mix, slide down edge of Eppendorf. Aids dissolving.
* Add yeast culture stock to all wells **last.**
* Do sulfoxaflor **last** as slightly different.

For All Insecticides Apart from Sulfoxaflor

Stock Solutions

1. **Make 200µl of 10mM stock solution** from 0.5M stock solution: 196µl media + 4µl 0.5 M stock solution.
2. **Make 200µl of 1mM stock solution** from 10mM stock solution: 180µl media + 20µl 10mM stock solution.
3. **Make 200µl of 0.01mM (10µM) stock solution** from 1mM stock solution: 198µl media + 2µl 1mM stock solution.

Well Construction

1. Using 10mM stock solution create **200µl** **5mM final well concentration**: 100µl 10mM stock solution + 58µl media + 2µl other solvent + 40µl 0.05OD yeast culture stock.
2. Using 10mM stock solution create **200µl 1mM final well concentration**: 136.4µl media + 20µl 10mM stock solution + 2µl other solvent + 1.6µl insecticide solvent + 40µl 0.05OD yeast culture stock.
3. Using 1mM stock solution create 800µl of **0.125mM stock solution** (with 1.25% solvent concentrations): 680.2µl media + 100µl 1mM stock solution + 10µl other solvent + 9.8µl insecticide solvent.
   * 1. Take 160µl of **0.125mM stock solution** and add 40µl 0.05OD yeast culture stock to create **200µl 0.1mM (100µM) final well concentration**.
4. Using 1mM stock solution create **200µl 0.01mM (10µM) final well concentration**: 154µl media + 2µl 1mM stock + 2µl other solvent + 2µl insecticide solvent + 40µl 0.05OD yeast culture stock.
5. Using 10µM stock solution create **200µl 0.001µM (1µM) final well concentration**: 136µl media + 20µl of 10µM stock solution + 2µl other solvent +2µl insecticide solvent + 40µl 0.05OD yeast culture stock.
6. Control – 156µl media + 2µl DMSO + 2µl methanol + 40µl 0.05OD yeast culture stock.

For Sulfoxaflor

Stock Solutions

1. **Make 200µl of 10mM stock solution** from 0.25M stock solution: 192µl media + 8µl 0.25M stock solution.
2. **Make 200µl of 1mM stock solution** from 10mM stock solution: 180µl media + 20µl 10mM stock solution.
3. **Make 200µl of 0.01mM (10µM) stock solution** from 1mM stock solution: 198µl + media 2µl 1mM stock solution.

Well Construction

1. Using 10mM stock solution create **200µl 2.5mM final well concentration**: 108µl media + 50µl 10mM stock solution + 2µl DMSO + 40µl 0.05OD yeast culture stock.
2. Using 10mM stock solution create **200µl 1mM final well concentration**: 136.8µl media + 20µl 10mM stock solution + 2µl DMSO + 1.2µl MeOH + 40µl 0.05OD yeast culture stock.
3. Using 1mM stock solution create 800µl of **0.125mM stock solution** (with 1.25% solvent concentrations): 680.4µl media + 100µl 1mM stock solution + 10µl DMSO + 9.6µl MeOH.
   * 1. Take 160µl of **0.125mM stock solution** and add 40µl 0.05OD yeast culture stock to create **200µl 0.1mM (100µM) final well concentration**.
4. Using 1mM stock solution create **200µl 0.01mM (10µM) final well concentration**: 154µl media + 2µl 1mM stock + 2µl DMSO + 2µl MeOH + 40µl 0.05OD yeast culture stock.
5. Using 10µM stock solution create **200µl 0.001mM (1µM) final well concentration**: 136µl media + 20µl of 10µM stock solution + 2µl DMSO +2µl MeOH + 40µl 0.05OD yeast culture stock.
6. Control – 156µl media + 2µl DMSO + 2µl methanol + 40µl 0.05OD yeast culture stock.

FILL PERIMETER WELLS WITH MEDIA

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