**Experiment 4 – Raman**

**Experimental Timeline:**

* Between 06-09 of April, groups 1 – 4 were tested.
* Which gives us 5 – 8 to be done.

**Material**

* Paper discs
* PDB 10% solution
* PDB 5% solution
* *A. alternata* suspensions
* Fresh seeds
* Hydrogen peroxide
* Petri dishes

**Experimental Groups:**

1. **Healthy seeds** (no visible infection)
2. **Infected seeds** with *Alternaria alternata* (mild)
3. **Infected seeds** with *Alternaria alternata* (moderate)
4. **Infected seeds** with *Alternaria alternata* (severe)
5. Paper discs soaked in **10% PDB and 5% PDB (Potato Dextrose Broth)**.
6. Paper discs soaked in **5% PDB + Alternaria** (mild, moderate, and severe infection levels).
7. Seeds treated with **hydrogen peroxide (H₂O₂)**—to test if the signal is related to oxidative processes potentially induced by ROS secreted by the fungus.
8. Paper discs soaked in **5% PDB + H₂O₂** (control for H₂O₂ treatment).

**Methods**

* Group 5: Six discs will be dipped in PDB solution (10% and 5%), three in each, and inserted to a disposable petri dish.
* Group 6: Nine discs, three for each stage (mild, moderate and severe), every group will be dipped into a solution containing PDB solution 5% + *Alternaria alternata* suspension, as shown in the table below:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Mild | Moderate | Severe |
| PDB volume (mL) | 5 | 5 | 5 |
| Suspension Concentration (conideas/mL) | 105 | 106 | 107 |
| Suspension Volume (mL) | 5 | 5 | 5 |

* Group 7: Fresh peppers will be opened on the spot, 20 seed will be extracted for each concentration of H2O2, as shown in the table below:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Low | Medium | High |
| H2O Volume (µL) | 19988.5 | 19977 | 19954 |
| H2O2 Volume (µL) | 11.5 | 23 | 46 |
| Concentration (mM) | 5 | 10 | 20 |

[(Jamaludin et al., 2020)](https://doi.org/10.3390/agronomy10040497)

* Group 8: Soak 3 discs in PDB 5% solution + H2O2 in each concentration (nine discs total).

**Hydrogen Peroxide Treatment Notes:**

* **Use fresh** hydrogen peroxide from the fridge.
* Sensitive to **light and heat**: wrap in **aluminum foil** and **keep on ice**.
* Apply **concentrated H₂O₂** **right before measurement**, let it sit for a few minutes, and then measure.
* Optionally, **leave the seeds** and **re-measure** after a few hours or the next day to observe progression.