Assessment of induced allelopathy in crop-weed co-culture with rye-pigweed model

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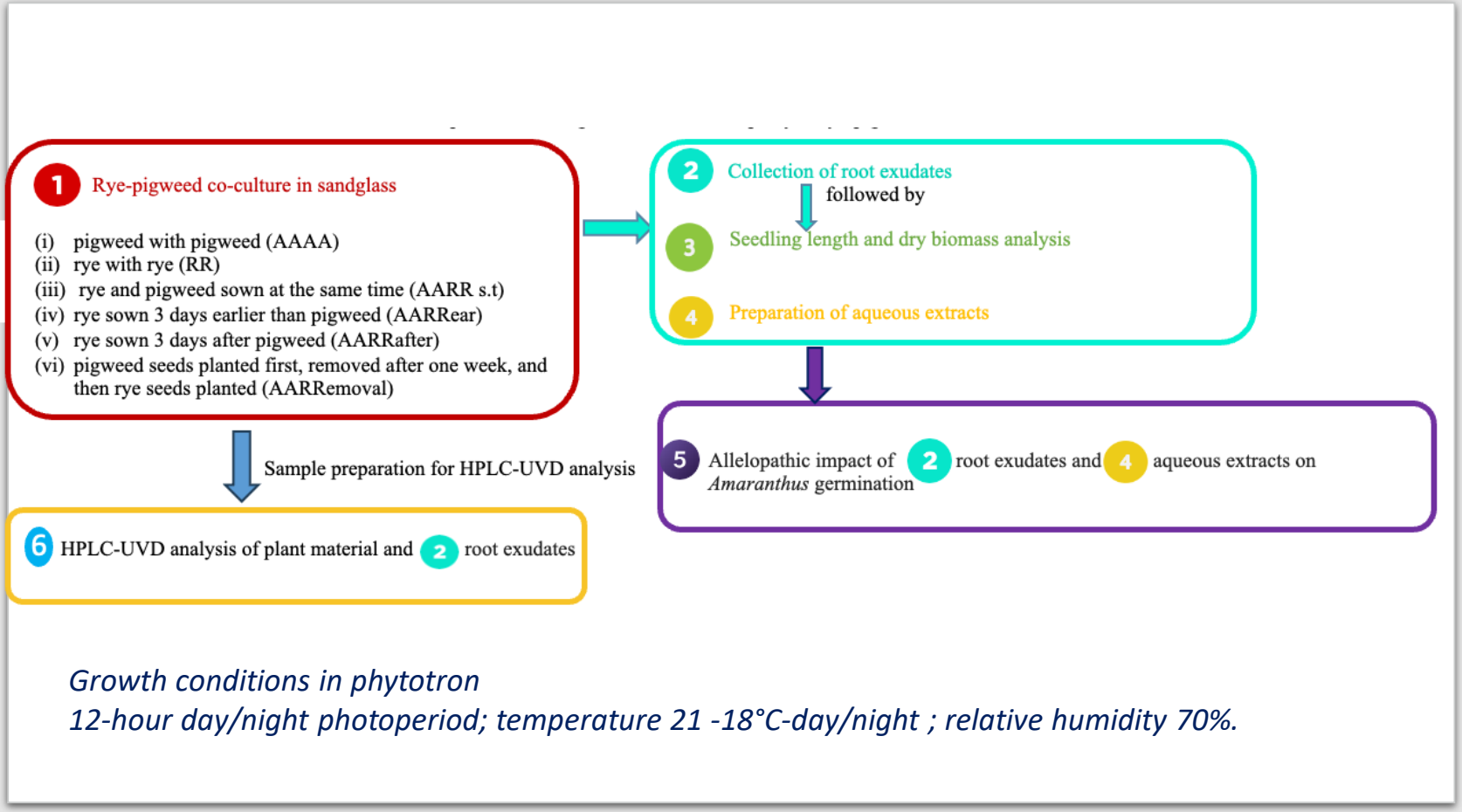
- **1. Introduction**
- What is allelopathy?

Objectives

- Determine conditions where Amaranthus growth is suppressed most by Rye or when Rye imposes its maximum allelopathic potential in Rye-Amaranthus co-culture
- To find the co-culture modality in which pigweed induces allelopathy in rye
- Assess the impact of the sowing time of seeds on allelopathy
- Determine conditions in which Rye detects its neighbours at the earliest
- Explore the root uptake of BXs by pigweed

- **2. Experimental Design**
- **3. Methodology**
- **4. Results and Discussion**
- **5. Conclusion**

2. Experimental Design



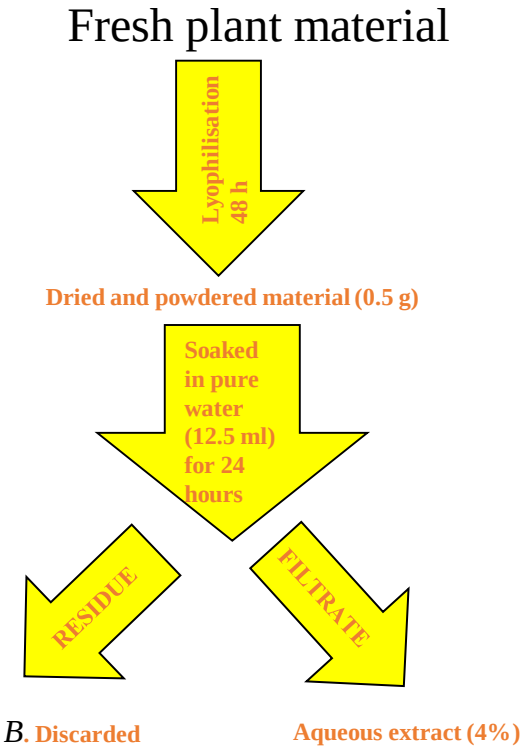
Growth conditions in phytotron
12-hour day/night photoperiod; temperature 21 -18°C-day/night ; relative humidity 70%.

3. Methodology

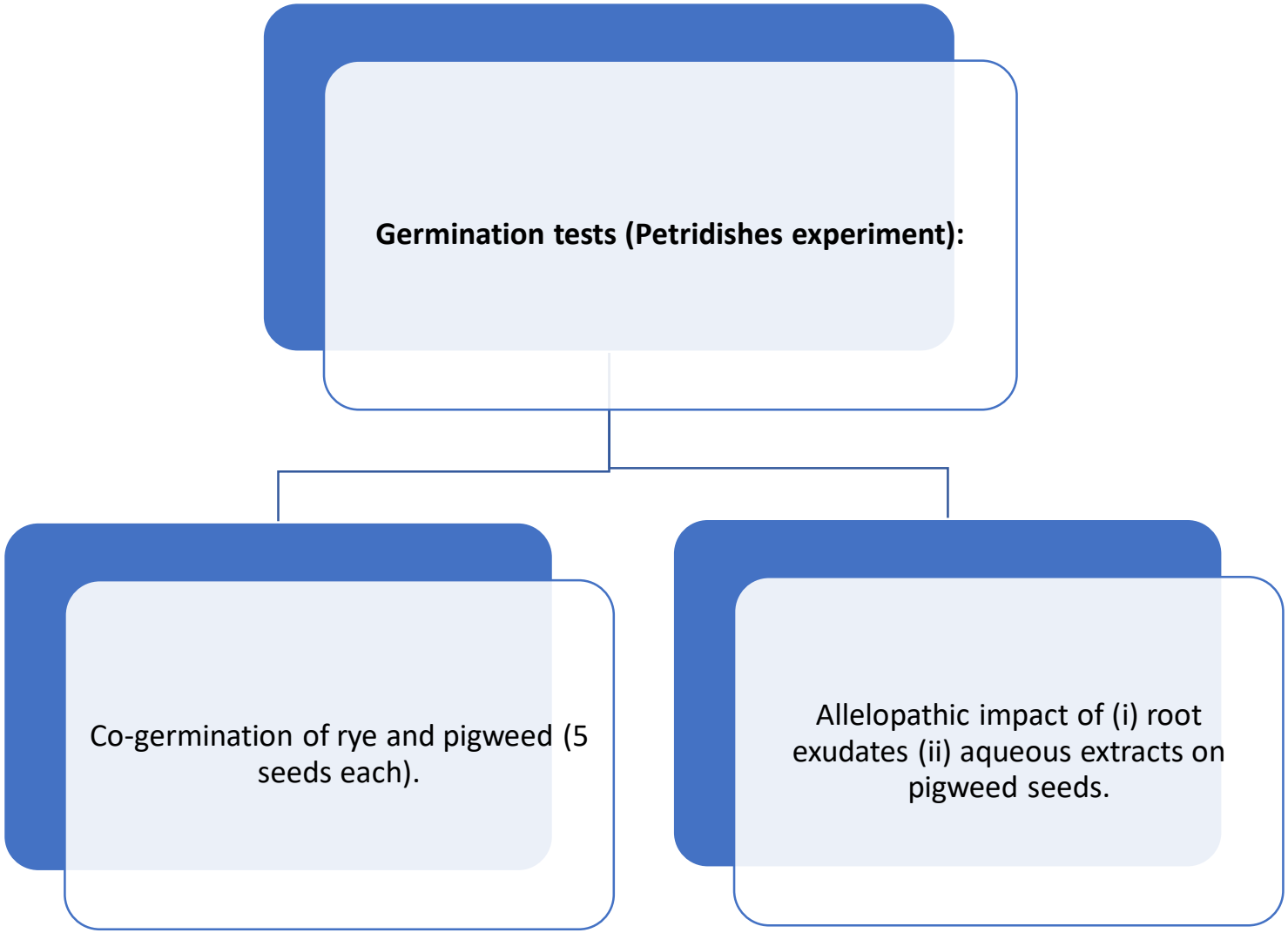
Modalities	Types of aqueous Extracts	Types of Exudates
1. AAAA (Amaranthus growing with Amaranthus)	ET1	ED1
2. **RR** (Rye growing with Rye)	ET2	ED2
3. **AARR s.t** (Rye, Amaranthus were sowed at the same time)	ET3	ED3
4. **AARRear** (Rye sown 3 days earlier than Amaranthus)	ET4	ED4
5. **AARRafter** (Rye sown 3 days after Amaranthus)	ET5	ED5
6. **AARRemoval** (In a plastic tube, we start by planting Amaranthus seeds, remove them after one week, and then plant Rye seeds, with the day of Rye planting considered as day 1.)	ET6	ED6

*Blank (sand serves as blank or control); Sampling time: 2 and 4 weeks

A. Customised device for root-exudate extraction



3. Methodology



3. Methodology

Benzoxazinoid extraction

freeze-dried plant material crushed ➞ 25 mg in Eppendorf tubes + 1 mL of extraction solvent (methanol/water/formic acid; 50/50/1; v/v/v) and 4 glass beads (<1 mm).

Extraction time 1 h in a Heindolph Multireax Agitator set at 2000 rpm

Separation of solid phase in an Eppendorf MiniSpin centrifuge for 8 minutes at maximum rpm (13,400)

The supernatant is syringed and filtered in a 0.45 µm PTFE filter placed in a vial and finally stored at 4 °C.

HPLC analysis (Agilent 1200 HPLC System)
Agilent Poroshell C18 column

Solution A (methanol/water/ortho-phosphoric acid 85%; 10/90/0.1; v/v/v)

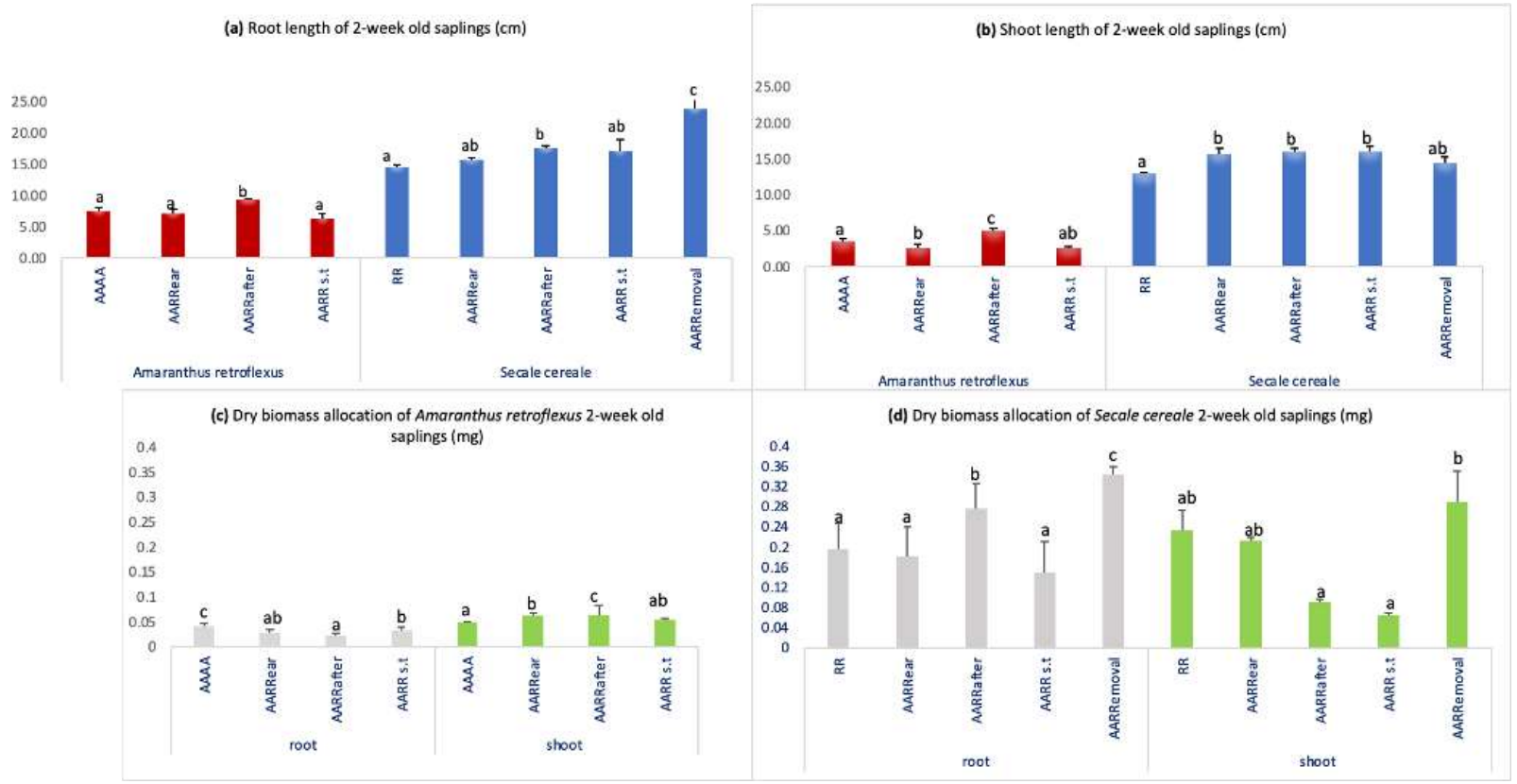
Solution B (methanol/ ortho-phosphoric acid 85% 100/0.1; v/v).

4. Results

- Growth parameters (root length, shoot length and dry biomass) separately* in all modalities after 2 and 4 weeks
- Germination indices of 2 and 4-week samples on exposure to exudates and extracts
- HPLC analysis of exudates and plant material (root and shoot separately* in all modalities) after 2 and 4 weeks. Standards used bezoxazinoids (DIBOA, DIMBOA and MBOA)

*for example in AARR modality, Amaranthus root and shoot are evaluated separately, so is Rye root and shoot

4.1. Growth parameters



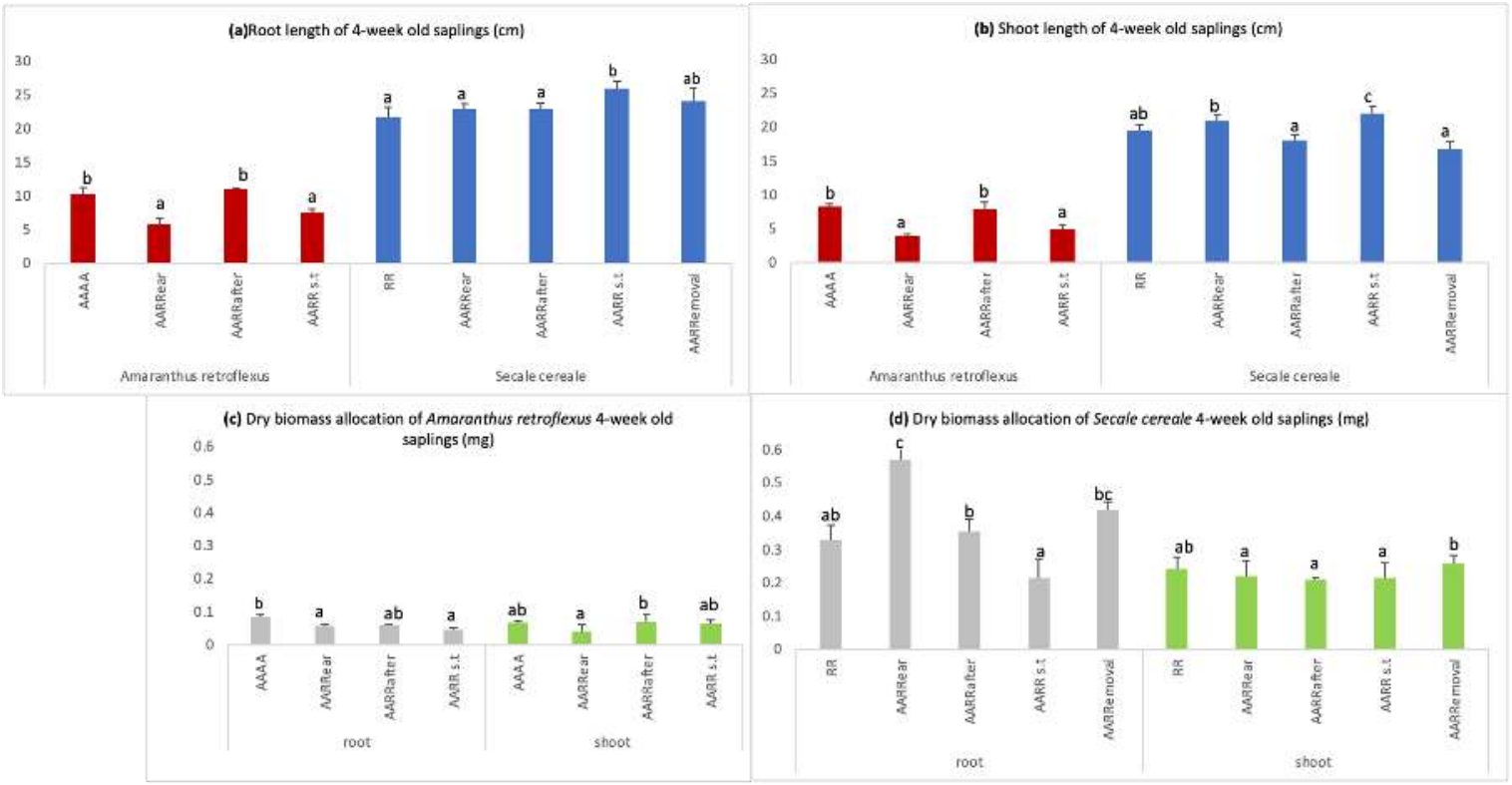
- There is no difference in pigweed root length in any modality except AARRafter (Fig. a).
  Surprisingly, AARRafter shows improved growth (both root and shoot) (Fig. a,b)

- Rye shows improved seedling length in all modalities compared to the control RR (Fig. a,b) with AARRemoval showing maximum root growth (Fig. a)

- Reduction in dry biomass of Amaranthus root in all modalities. However, the shoot shows improved biomass particularly AARRafter when compared to the control.

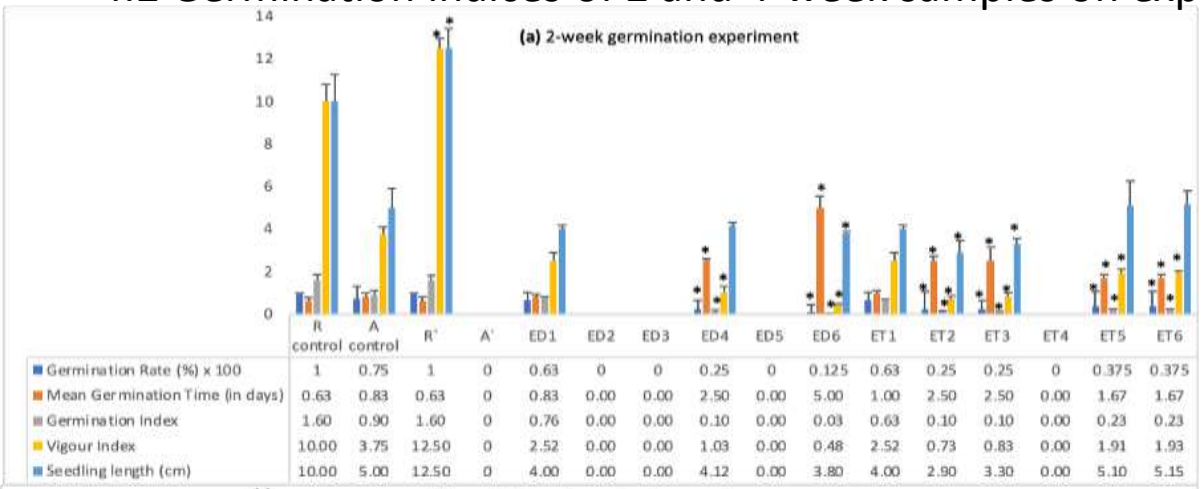
- The trend is different in Rye. Rye in AARRemoval shows the highest shoot/root biomass.

4.1. Growth parameters

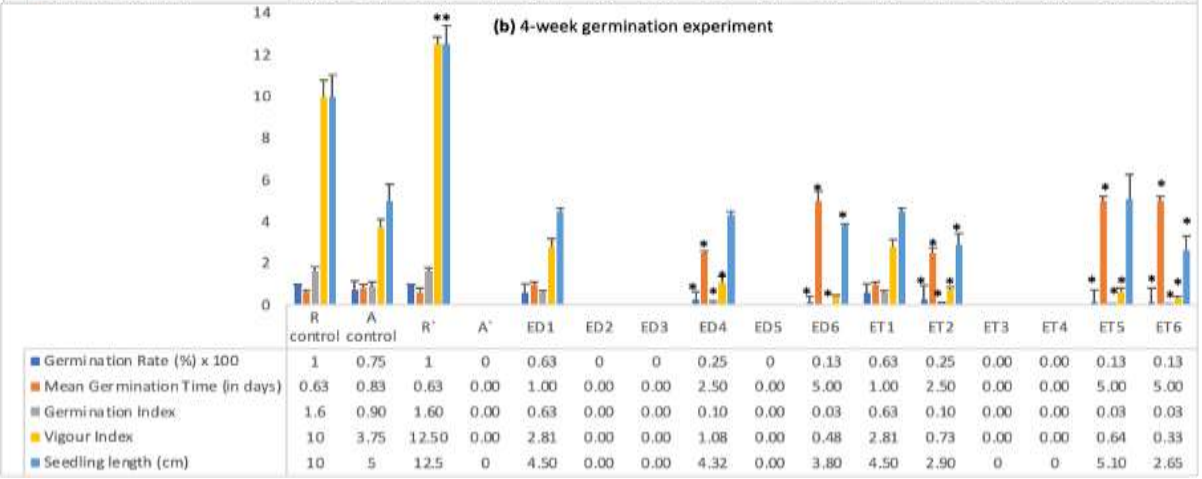


- Reduction in pigweed seedling length is observed in all modalities except AARRafter. It follows the same trend as at 2-week old stage.
- Rye did not show any major changes in root length except AARR s.t showing improved growth (Fig. a). Moreover AARRs.t shows maximum growth (Fig. a, b).
- Reduction in dry biomass of pigweed root was observed in all modalities except AARRafter.
- Rye in AARRear shows the highest root biomass followed by AARRemoval in contrast to the control (Fig. 2d). Similarly, AARRemoval (and control) shows the highest shoot biomass.

4.2 Germination indices of 2 and 4-week samples on exposure to exudates and extracts



	R control	A control	R'	A'	ED1	ED2	ED3	ED4	ED5	ED6	ET1	ET2	ET3	ET4	ET5	ET6
Germination Rate (%) x 100	1	0.75	1	0	0.63	0	0	0.25	0	0.125	0.63	0.25	0.25	0	0.375	0.375
Mean Germination Time (in days)	0.63	0.83	0.63	0	0.83	0.00	0.00	2.50	0.00	5.00	1.00	2.50	2.50	0.00	1.67	1.67
Germination Index	1.60	0.90	1.60	0	0.76	0.00	0.00	0.10	0.00	0.03	0.63	0.10	0.10	0.00	0.23	0.23
Vigour Index	10.00	3.75	12.50	0	2.52	0.00	0.00	1.03	0.00	0.48	2.52	0.73	0.83	0.00	1.91	1.93
Seedling length (cm)	10.00	5.00	12.50	0	4.00	0.00	0.00	4.12	0.00	3.80	4.00	2.90	3.30	0.00	5.10	5.15



	R control	A control	R'	A'	ED1	ED2	ED3	ED4	ED5	ED6	ET1	ET2	ET3	ET4	ET5	ET6
Germination Rate (%) x 100	1	0.75	1	0	0.63	0	0	0.25	0	0.13	0.63	0.25	0.00	0.00	0.13	0.13
Mean Germination Time (in days)	0.63	0.83	0.63	0.00	1.00	0.00	0.00	2.50	0.00	5.00	1.00	2.50	0.00	0.00	5.00	5.00
Germination Index	1.6	0.90	1.60	0.00	0.63	0.00	0.00	0.10	0.00	0.03	0.63	0.10	0.00	0.00	0.03	0.03
Vigour Index	10	3.75	12.50	0.00	2.81	0.00	0.00	1.08	0.00	0.48	2.81	0.73	0.00	0.00	0.64	0.33
Seedling length (cm)	10	5	12.5	0	4.50	0.00	0.00	4.32	0.00	3.80	4.50	2.90	0	0	5.10	2.65

- Pigweed shows no germination when exposed to ED2, ED3, ED5, ET1 and ET4
- Pigweed shows the highest GR in control (75%)
- MGT tends to increase while GI and VI decrease
- Pigweed shows no germination when exposed to exudates (ED2, ED3, ED5) and extracts (ET3, ET4
- plant extracts from 4-week-old samples have stronger inhibitory potential

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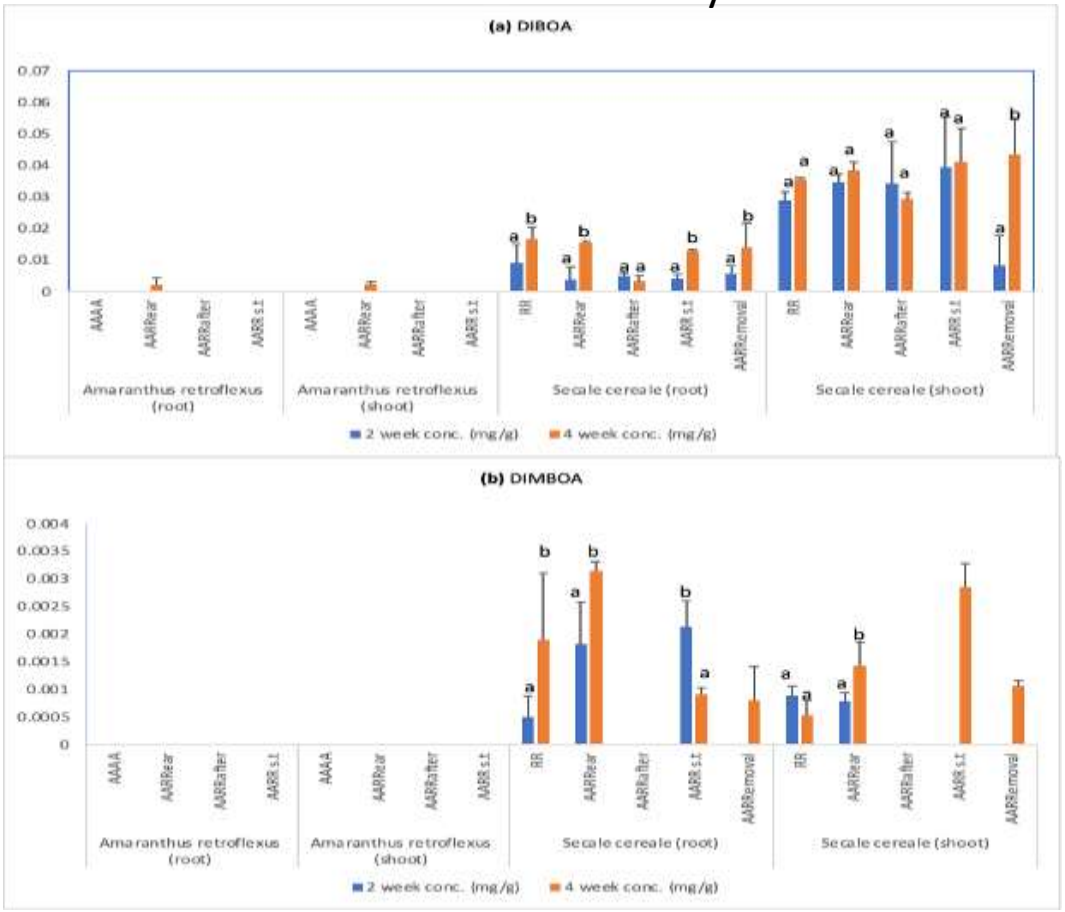
- MGT, GI and VI follow a similar trend as observed earlier

- In the co-germination test, rye shows a 100% GR both in control (R control) and in the presence of pigweed (R') (Fig. a,b).
- Moreover, rye shows improved VI and SL in the presence of pigweed.
- Pigweed did not germinate in rye presence of rye (A').

4.3. Benzoxazinoid analysis



- No BXs (DIBOA, DIMBOA and MBOA) were detected in root exudates
- In the plant material, there were no BXs detected in the control group of pigweed (AAAA).
- MBOA not detected
- DIBOA (shown in Fig. a) was found in all 2-week and 4-week-old rye plants
- Significant increase in the DIBOA in 4-week-old root samples
- “AARRemoval” had the highest DIBOA in the shoots at the 4-week-old stage.
- DIBOA was detected in some 4-week-old pigweed samples in the modality “AARRear”.

5. Conclusion

- Pigweed experiences a significant reduction in growth in rye's presence, implying allelopathic effects.
- Contrastingly, rye exhibits increased seedling length and BXs upsurge in response to pigweed presence.
- HPLC-DAD analysis identifies allelopathic compounds (BXs), 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) in the rye.
- Allelopathy in rye-pigweed co-cultures is influenced by seed timing and age-dependent dynamics of plants' allelopathic compounds

Thank You
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Allelopathy Team

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