Light Crude Oil Contamination Suppresses Seed Germination And Early Seedling Development In Amaranthus Hybridus L.: A Concentration-Dependent Phytotoxicity Assessment

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ABSTRACT

Petroleum hydrocarbon (PHC) contamination poses significant risks to agricultural productivity and food safety in oil-producing regions. This study examined the phytotoxic effects of light crude oil (0.0–10.0% v/v) on the germination and early seedling growth of Amaranthus hybridus L. using a controlled Petri-dish bioassay. Seed germination, germination velocity indices, biomass, and vigor metrics were analyzed using one-way ANOVA followed by Tukey's HSD tests (n = 6). Germination percentage decreased significantly at concentrations above 1.0% v/v ($P < 0.001$) and was entirely inhibited at 10.0% v/v. Higher oil concentrations significantly and dose-dependently reduced all germination velocity indices, seedling emergence, vigor, tolerance indices, seedling length, and fresh weight (all $P < 0.001$). In contrast, dry weight was not affected, and relative water content declined only at the highest concentration (10.0% v/v, $P < 0.01$).

In summary, light crude oil demonstrates pronounced phytotoxicity toward A. hybridus, with germination being more adversely affected than post-emergence dry biomass accumulation. Because of its high sensitivity, A. hybridus serves as an effective bioindicator of crude oil contamination in agricultural soils and a valuable model for regional risk assessments.

Keywords: Amaranthus hybridus; light crude oil; seed germination; phytotoxicity; petroleum hydrocarbon; seedling vigor; bioindicator; concentration-response

1. Introduction

1.1 Research Context

Contamination of agricultural soils by petroleum hydrocarbons (PHCs) is a pervasive and growing environmental concern, especially in regions with active oil exploration, production, and transport infrastructure. Crude oil spills introduce a chemically complex mixture of aliphatic and aromatic compounds, asphaltenes, and non-hydrocarbon residues into the soil matrix. The consequences include disruption of soil physicochemical properties, modification of microbial communities, depletion of plant-available nutrients (particularly nitrogen and phosphorus), and accumulation of phytotoxic constituents including polycyclic aromatic hydrocarbons (PAHs) and aromatic fractions such as benzene, toluene, ethylbenzene, and xylene (BTEX). These alterations collectively compromise soil capacity to sustain plant growth (Haider et al., 2021; Mekonnen et al., 2024). Dose-dependent impacts of light crude oil contamination on seed germination and seedling growth in Zea mays L. (maize), a critical agricultural species has been studied by Alzway etal. in 2025, as per their study, increasing concentrations of light crude oil in soil progressively suppress germination kinetics and reduce seedling vigor.

Plant species vary considerably in their tolerance to PHC-contaminated soils, and this variation is both species-specific and concentration-dependent. Some species exhibit partial tolerance through exclusion mechanisms, enhanced antioxidant enzyme activity, or rhizosphere-mediated hydrocarbon degradation (Xu & Johnson, 1995; Mekonnen et al., 2024). In contrast, shallow-rooted leafy vegetables tend to be acutely sensitive to even moderate PHC concentrations, making them valuable subjects for phytotoxicity assessments (Luo et al., 2024; Almaroai & Eissa, 2025).

1.2 The Study Species: Amaranthus hybridus L.

Amaranthus hybridus L. (Amaranthaceae), known as the Never-fading flower or Green amaranth, is a widely cultivated leafy vegetable consumed across Africa, Asia, and the Mediterranean. Rich in protein, essential amino acids, minerals, and antioxidants, it is commonly found in waste grounds, cultivated fields, and roadsides, frequently in proximity to oil infrastructure. It is documented in traditional medicine for treating intestinal bleeding, diarrhoea, and excessive menstruation (Foster & Duke, 1990; Moerman, 1998), and a tea from its leaves is considered astringent (Tanaka, 1976; Edeoga & Otoide, 2001). Its ecological ubiquity in oil-producing regions of Africa and the Middle East makes it an important candidate for phytotoxicity investigations.

Germination of A. hybridus is particularly susceptible to oil-contaminated soils; only a fraction of seeds sown in contaminated media germinate, and those that do exhibit markedly delayed emergence (Azizi & Fuji, 2006; Merkl et al., 2005, Alzway & Mansour,2024). However, systematic multiparameter evaluation across a wide range of defined light crude oil concentrations has not been previously undertaken for this species.

1.3 Introduction to the Literature

A substantial body of literature documents the inhibitory effects of crude oil and refined petroleum products on plant germination and growth. Early studies by (De Jong, 1980) and (Udo & Fayemi, 1975) established that crude oil spills reduce soil nutrient availability and impair cereal germination. Subsequent work by (Quinones-Aquilar et al., 2003) demonstrated concentration-dependent delays in maize emergence in crude oil-polluted soils, while (Agbogidi et al., 2007) showed that the timing of contamination substantially modulates growth inhibition severity in Zea mays (Odjegba & Sadiq, 2002) specifically documented that spent engine oil significantly reduced germination

percentage, chlorophyll content, and protein levels in A. hybridus, providing a species-specific baseline directly relevant to the present investigation.

More recent systematic reviews have refined mechanistic understanding. (Haider et al., 2021), publishing in Environmental Research (Q1, Elsevier), identified three principal PHC phytotoxicity pathways: inhibition of water absorption due to hydrophobic soil-particle coating, induction of reactive oxygen species (ROS)-mediated oxidative stress, and direct membrane and organelle damage in seed cells. These mechanisms are corroborated by (Luo et al., 2024), publishing in the Journal of Hazardous Materials (Q1, Elsevier), who confirmed that PHCs rank among the most potent inhibitors of seed germination across crop species. Critically, germination inhibition is now recognised as a more sensitive early-response indicator than biomass loss.

At the regional level, Anoliefo and Edegbai, 2000, reported that spent oil significantly impaired growth in Solanum species, and (Daniel-Kalio & Pepple, 2006,Hamaden.G.,2021) documented growth inhibition in Commelina benghalensis following bonny light crude oil pollution, highlighting the relevance of light crude fractions to vegetable crop phytotoxicity in African agricultural systems.

1.4 Research Gaps and Objectives

Despite accumulated evidence on PHC phytotoxicity, critical gaps persist. First, most published germination bioassays have used spent engine oil or undifferentiated total petroleum hydrocarbon (TPH) soil mixtures rather than light crude oil at defined volumetric concentrations, limiting mechanistic resolution. Second, no study has simultaneously quantified the full suite of germination velocity indices (GR, MDG, MGT, GI, CGV) alongside the complete panel of seedling development parameters (SE%, SVI, TI, RWC%, seedling length, fresh and dry weight) for A. hybridus under graded light crude oil exposure. Third, while phytotoxicity tests using germination endpoints are widely endorsed as short-term toxicity screening tools (Masakorala et al., 2013; Almaroai & Eissa, 2025), no study has formally evaluated A. hybridus as a candidate bioindicator benchmarked against light crude oil concentrations spanning the full range from subinhibitory (0.5% v/v) to complete inhibition (10.0% v/v).

The objectives of the present study were therefore: (i) to quantify the concentration-dependent effects of light crude oil on germination parameters of A. hybridus; (ii) to assess the relative sensitivity of germination-stage versus seedling-growth-stage responses; and (iii) to evaluate the potential of A. hybridus as a bioindicator for light crude oil contamination in agricultural soils.

2. Literature Review

2.1 Complementary Perspectives on PHC Phytotoxicity

Research on PHC phytotoxicity converges on two complementary mechanistic frameworks. The first is a soil-centred perspective, in which PHC contamination alters the physical, chemical, and biological properties of the soil medium. PHC molecules adsorb to soil particles, forming hydrophobic films that disrupt water-retention capacity and reduce hydraulic conductivity (Mekonnen et al., 2024). This physical alteration elevates the C:N ratio, immobilises phosphorus and potassium, and suppresses nitrification and ammonification processes (Haider et al., 2021), creating conditions unfavourable for seed imbibition and radicle emergence.

The second is a plant-centred perspective, in which PHC compounds exert biochemical toxicity directly on seed and seedling tissues. Low-molecular-weight aromatic compounds particularly BTEX and PAH fractions prevalent in light crude oil are lipophilic and penetrate seed coat and cell membranes, disrupting membrane integrity, inhibiting enzymatic

activity, and inducing oxidative stress through ROS accumulation (Luo et al., 2024; Haider et al., 2021). Both frameworks are relevant to interpreting the present findings, as the Petri-dish bioassay integrates both direct chemical exposure and indirect effects on seed water availability.

2.2 Key Literature Summary

Table 1 provides a structured overview of the most relevant studies underpinning the present investigation.

Author(s) & Year	Study Focus	Key Finding	Relevance
Odjegba & Sadiq (2002)	Spent engine oil effects on A. hybridus	Significant reductions in germination %, chlorophyll, and protein	Direct species-level baseline
Quinones-Aquilar et al. (2003)	Crude oil and maize emergence/growth	Concentration-dependent delays in emergence	Cross-species comparison
Agbogidi et al. (2007)	Timing of crude oil on Zea mays	Earlier application caused greater inhibition	Temporal context
Haider et al. (2021) [Q1]	Review: PHC phytotoxicity mechanisms	PHs reduce germination; induce oxidative stress	Mechanistic framework
Luo et al. (2024) [Q1]	Environmental risk substances on germination	Three mechanisms: water blockage, oxidative damage, cell injury	Mechanistic interpretation
Almaroai & Eissa (2025) [Q1]	PHC sludge on Salicornia	Concentration-dependent germination suppression	Validates study design
Mekonnen et al. (2024) [Q1]	PHC soil bioremediation review	PHC coating disrupts moisture retention	Soil-physical mechanism
Masakorala et al. (2013) [Q2]	Long-term TPH soil phytotoxicity	Germination more sensitive than biomass	Differential sensitivity support
Ossai et al. (2024) [Q2]	Ecotoxicology of waste engine oils	PHs impair growth and physiology multi-species	Broader ecotoxicological context

Table 1. Summary of key literature and relevance to the present investigation. PHC = petroleum hydrocarbons; [Q1/Q2] = journal quartile ranking.

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.3 Critical Review and Literature Gaps

The literature consistently indicates that germination endpoints are more sensitive to PHC contamination than are biomass endpoints (Luo et al., 2024; Masakorala et al., 2013), a pattern the present results corroborate. The use

of a defined light crude oil at volumetric concentrations (% v/v) offers greater mechanistic precision than studies employing spent engine oil, as it isolates the phytotoxic contribution of the light crude fraction specifically. Three persistent gaps in the existing literature justify the present research: (1) absence of species-specific multiparameter data for A. hybridus at graded light crude oil concentrations; (2) no empirically established inhibition threshold for this species under light crude oil; and (3) no formal bioindicator evaluation for A. hybridus in light crude oil contexts.

The experimental design was shaped directly by the critical review. The Petri-dish germination bioassay follows the AOSA (AOSA, 1983) standard protocol applied in analogous phytotoxicity studies (Luo et al., 2024; Almaroai & Eissa, 2025). The concentration range (0.5-10.0% v/v) was designed to bracket the EC50 range for germination reported for other species in comparable bioassays (Masakorala et al., 2013), ensuring both subinhibitory and fully inhibitory concentrations were captured. The multiparameter index approach was adopted following recommendations by (Luo et al., 2024) and (Haider et al., 2021) that single-endpoint assessments systematically under-represent phytotoxic effects.

3. Materials and Methods

3.1 Plant Material and Chemicals

Seeds of Amaranthus hybridus L. (Amaranthaceae) were collected from the Hei Al-Salam area and stored at ambient temperature (25-30 degrees C) prior to use. Light crude oil was obtained from Alberigh port, Alamal field, and applied at volumetric concentrations of 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0% (v/v). Seeds were surface-sterilised with 10% formaldehyde solution for five minutes prior to plating.

3.2 Experimental Procedure

Sterilised glass Petri dishes (9.0 cm diameter) lined with double layers of Whatman No. 1 filter paper were used. Dishes were oven-sterilised at 180 degrees C for two hours. Five seeds were placed in each dish, with six replicates per treatment (n = 6 per concentration level). Filter papers were moistened with 3 mL of distilled water (control) or the corresponding crude oil test solution. All dishes were incubated at 20 degrees C in a Gallenkamp incubator for one week, with moisture replenished uniformly as required.

3.3 Germination Parameters

Germination percentage (GP%) was calculated as (germinated/sown) x 100 (Ruan et al., 2002). Mean germination time (MGT) was computed as sum(ni x di) / N, where ni is the number germinating on day i and N is total germinated (Ellis & Roberts, 1981; Alvarado et al., 1987; Lafond & Baker, 1986). Mean daily germination (MDG) was calculated as final GP% / total observation days (Czabator, 1962; Scott et al., 1984). Germination rate (GR) was calculated as emerged seeds / days after planting (Rastegar et al., 2011). The coefficient of germination velocity (CGV) was calculated following (Maguire, 1962). The germination index (GI) was calculated as (Gs/Gc) x (Ls/Lc) x 100, where subscripts s and c denote sample and control respectively (AOSA, 1983). Inhibition of final germination percentage (IFG%) was calculated as (non-germinated / total) x 100.

3.4 Seedling Development Parameters

Germinated seeds were allowed to develop for an additional two weeks. Seedling length (cm), fresh weight (g), and dry weight (g) were recorded for ten seedlings per replicate. Relative water content (RWC%) was calculated as [(FW - DW) / FW] x 100. Seedling emergence percentage (SE%) was calculated as (emerged / sown) x 100. Seedling vigor index (SVI) was computed as: seedling length (cm) x final germination percentage. Tolerance index (TI)

was calculated as: seedling length in treatment / seedling length in control.

3.5 Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS v.26 (IBM Corp., Armonk, NY, USA). Where significant treatment effects were detected ($P < 0.05$), Tukey's honestly significant difference (HSD) post-hoc test was applied to identify pairwise differences among treatment means. Results are reported as means +/- standard error of the mean (SEM). Significance levels: + = not significant ($P >= 0.05$); ** = $P < 0.01$; *** = $P < 0.001$. Groups sharing the same letter code are not significantly different.

4. Results

4.1 Seed Germination

4.1.1 Daily Germination Percentage

No germination occurred on Day 1 across any treatment. From Day 2 onwards, a clear concentration-dependent inhibition pattern emerged (Table 2; Figure 1). At 0.5% v/v, germination progression was comparable to the control. At concentrations above 1.0% v/v, germination was significantly reduced relative to the control ($F = 13.60$, $P < 0.001$), and was completely abolished at 10.0% v/v. Tukey's pairwise comparisons confirmed significant differences between the control and all concentrations above 1.0% v/v on Days 4 and 7 (Day 4: $F = 12.48$, $P < 0.001$; Day 7: $F = 16.42$, $P < 0.001$).

Germination time course (Figure A). The kinetic trajectory across all seven days (Figure A) reveals that concentrations >= 6.0% v/v produced a flat, completely suppressed germination curve from Day 2 onward. The 2.0% v/v treatment exhibited a progressive rise from 10.0% on Day 2 to 43.3% by Day 7, distinguishing it behaviorally from both the control and fully inhibitory concentrations. This temporal pattern is not captured by single-day snapshots.

Trt (%)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.0	0.00+/-0.00	56.7a+/-6.70	56.7a+/-6.70	56.7a+/-6.70	63.3a+/-3.33	70.0a+/-5.80	70.0a+/-5.80
0.5	0.00+/-0.00	56.7a+/-12.0	66.7a+/-6.70	66.7a+/-6.70	70.0a+/-5.80	70.0a+/-5.80	70.0a+/-5.80
1.0	0.00+/-0.00	20.0b+/-5.80	26.7ac+/-3.33	26.7ac+/-3.33	26.7bc+/-3.33	26.7bc+/-3.33	26.7bc+/-3.33
2.0	0.00+/-0.00	10.0b+/-0.00	36.7ac+/-16.7	36.7ac+/-16.7	40.0ac+/-15.3	43.3ac+/-14.5	43.3ac+/-14.5
4.0	0.00+/-0.00	10.0b+/-10.0	20.0bc+/-5.80	20.0bc+/-5.80	23.3b+/-8.80	23.3bc+/-8.80	23.3bc+/-8.80
6.0	0.00+/-0.00	0.00b+/-0.00	0.00bc+/-0.00	0.00bc+/-0.00	3.33bc+/-3.33	3.33b+/-3.33	3.33b+/-3.33
8.0	0.00+/-0.00	3.33b+/-3.33	3.33bc+/-3.33	3.33bc+/-3.33	6.70b+/-3.33	6.67b+/-3.33	6.67b+/-3.33

10.0	0.00+/- 0.00	0.00b+/- 0.00	0.00bc+/- 0.00	0.00bc+/- 0.00	0.00bc+/- 0.00	0.00b+/- 0.00	0.00b+/- 0.00

Table 2. Effect of light crude oil dilutions on daily germination percentages (%) of Amaranthus hybridus L. seeds (mean +/- SEM; n = 6). Different superscript letters within a column indicate significant differences (Tukey's HSD). + = not significant (Day 1); *** = P < 0.001.

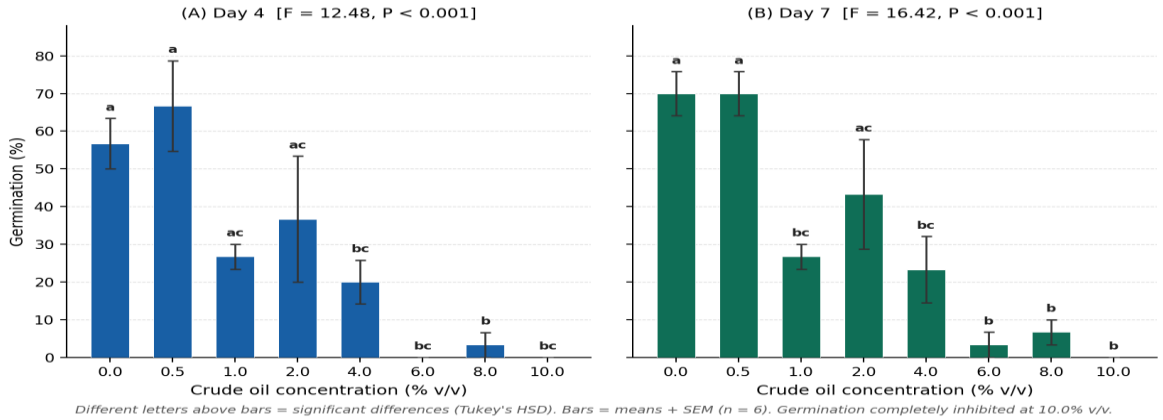

Figure 1. Effect of light crude oil dilutions on daily germination percentages (%) of Amaranthus hybridus L. seeds on Day 4 (panel A) and Day 7 (panel B). Bars = means + SEM (n = 6). Different letters above bars = significant differences (Tukey's HSD). *** = P < 0.001. Blue bars: Day 4 (F = 12.48); green bars: Day 7 (F = 16.42). Germination completely inhibited at 10.0% v/v.

Day 4 means: 0.0%: 56.7a; 0.5%: 66.7a; 1.0%: 26.7ac; 2.0%: 36.7ac; 4.0%: 20.0bc; 6.0%: 0.00bc; 8.0%: 3.33bc; 10.0%: 0.00bc. | Day 7 means: 0.0%: 70.0a; 0.5%: 70.0a; 1.0%: 26.7bc; 2.0%: 43.3ac; 4.0%: 23.3bc; 6.0%: 3.33b; 8.0%: 6.67b; 10.0%: 0.00b.

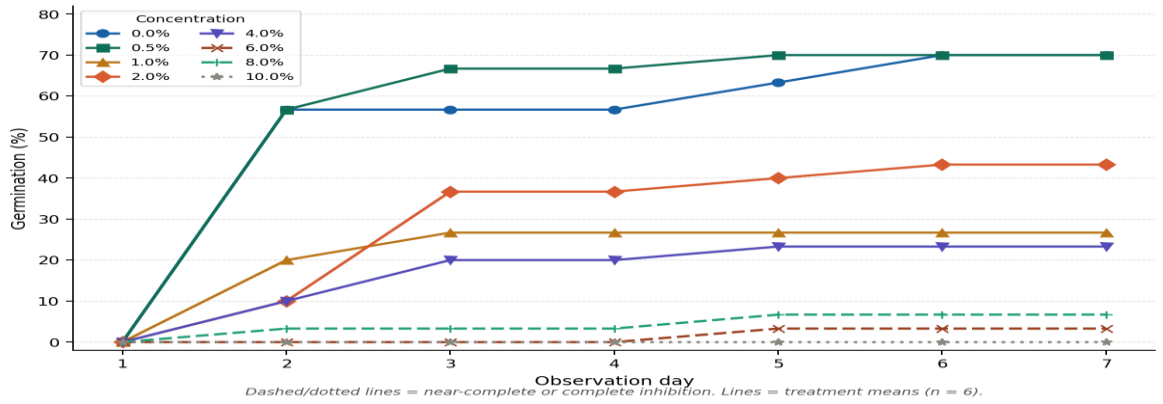

Figure A (Suggested). Germination time course of Amaranthus hybridus L. seeds across seven observation days for all eight light crude oil concentrations. Lines = treatment means (n = 6). Dashed/dotted lines indicate near-complete or complete inhibition (6.0-10.0% v/v). Note the progressive germination trajectory of the 2.0% v/v treatment from Day 2 to Day 7, distinguishable from both the control and fully inhibitory concentrations. Recommended insertion point: Section 4.1.1, after Table 1.

4.1.2 Inhibition of Final Germination Percentage

The inhibition of final germination percentage (IFG%) increased significantly with crude oil concentration ($F = 19.18$, $P < 0.001$; Figure 2).

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IFG% exceeded 50% at all concentrations >= 1.0% v/v and surpassed 90% at concentrations >= 6.0% v/v, reaching 100% at 10.0% v/v, confirming complete germination suppression at the highest concentration tested

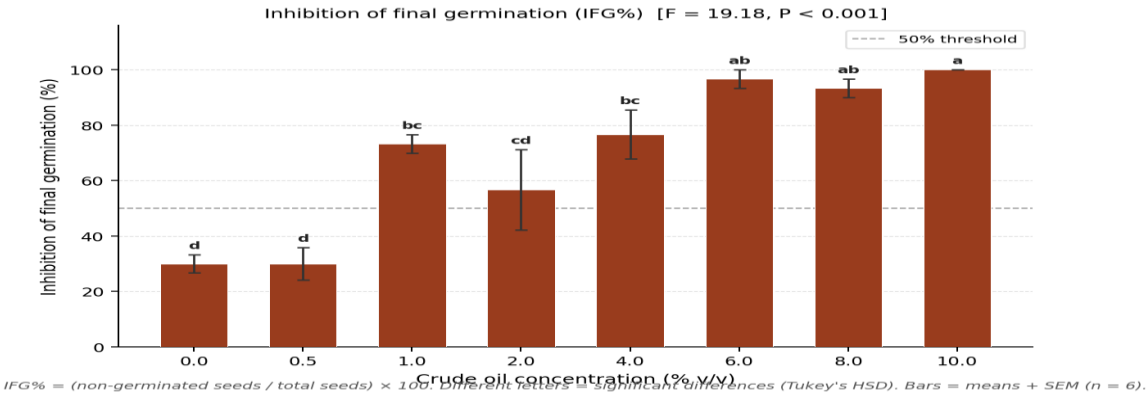

Figure 2. Effect of light crude oil dilutions on inhibition of final germination percentage (IFG%) of Amaranthus hybridus L. seeds. IFG% = (non-germinated / total seeds) x 100. Bars = means + SEM (n = 6). *** = $P < 0.001$ ($F = 19.18$). Dashed line at 50% IFG% threshold. Different letters = significant differences (Tukey's HSD).

Data: 0.0%: 30.0d; 0.5%: 30.0d; 1.0%: 73.3bc; 2.0%: 56.7cd; 4.0%: 76.7bc; 6.0%: 96.7ab; 8.0%: 93.3ab; 10.0%: 100.0a.

4.1.3 Germination Velocity and Index Parameters

Significant reductions were observed in GR ($F = 19.18$, $P < 0.001$), MDG ($F = 16.42$, $P < 0.001$), MGT ($F = 18.05$, $P < 0.001$), and GI ($F = 7.52$, $P < 0.001$), all declining in a concentration-dependent manner above 1.0% v/v (Table 2; Figure B). The coefficient of germination velocity (CGV) was significantly lower at concentrations of 6.0% and 10.0% v/v relative to the control ($F = 14.27$, $P < 0.001$; Figure 3).

Multi-parameter normalised profile (Figure B). Figure B presents GR, MDG, MGT, and GI normalised to the control (= 100%) as a grouped bar chart. This visualisation confirms that all four indices collapse in parallel above 1.0% v/v none remaining above 50% of control at 4.0% v/v and collectively reach zero at 10.0% v/v, strengthening confidence in the robustness of the concentration-response relationship.

Trt (%)	GR (seeds day^{-1})	MDG (% day^{-1})	MGT (days)	GI (%)
0.0	1.09a +/- 0.05	10.0a +/- 0.80	25.5a +/- 0.90	100.0a +/- 0.00
0.5	1.00ac +/- 0.08	10.0a +/- 0.80	26.3a +/- 2.40	90.2ac +/- 14.6
1.0	0.40b +/- 0.05	3.80bd +/- 0.50	10.1bc +/- 1.20	15.95b +/- 9.99

2.0	0.62bc +/- 0.20	6.20ad +/- 2.08	14.9ac +/- 5.40	43.8bc +/- 25.8
4.0	0.33b +/- 0.13	3.33cd +/- 1.30	8.30bc +/- 3.10	11.6bc +/- 6.9
6.0	0.05bd +/- 0.05	0.50cd +/- 0.50	0.90b +/- 0.90	0.54b +/- 0.54
8.0	0.09bd +/- 0.05	0.95cd +/- 0.50	2.14b +/- 1.10	1.10b +/- 0.60
10.0	0.00bd +/- 0.00	0.00cd +/- 0.00	0.00b +/- 0.00	0.00b +/- 0.00

Table 2. Effect of light crude oil dilutions on germination rate (GR), mean daily germination (MDG), mean germination time (MGT), and germination index (GI) of Amaranthus hybridus L. seeds (mean +/- SEM; n = 6). *** = P < 0.001. Different letters = significant differences (Tukey's HSD).

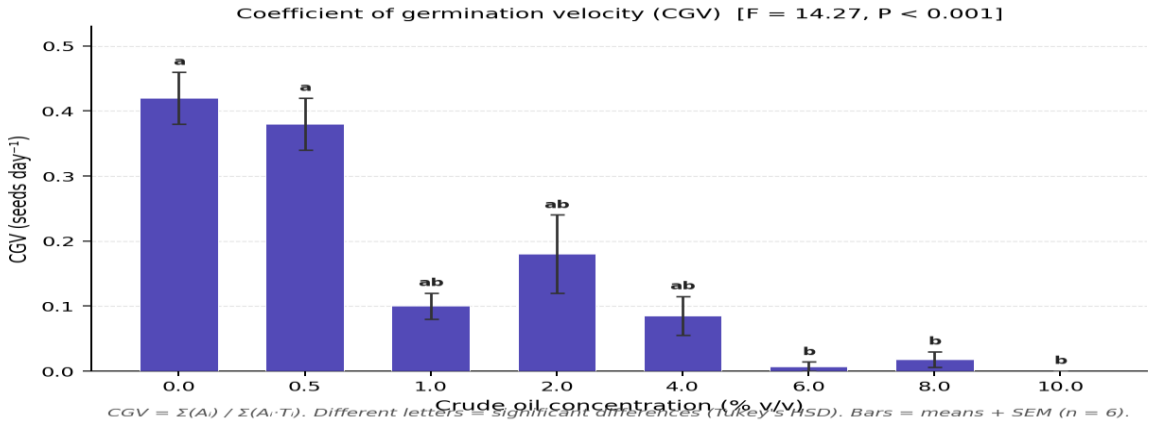

Figure 3. Effect of light crude oil dilutions on the coefficient of germination velocity (CGV) of Amaranthus hybridus L. seeds. CGV = sum(Ai) / sum(Ai x Ti). Bars = means + SEM (n = 6). *** = P < 0.001 (F = 14.27). Different letters = significant differences (Tukey's HSD).

CGV significantly lower at 6.0% and 10.0% v/v vs. control (Tukey's HSD). No significant differences detected between control and 1.0-4.0% v/v.

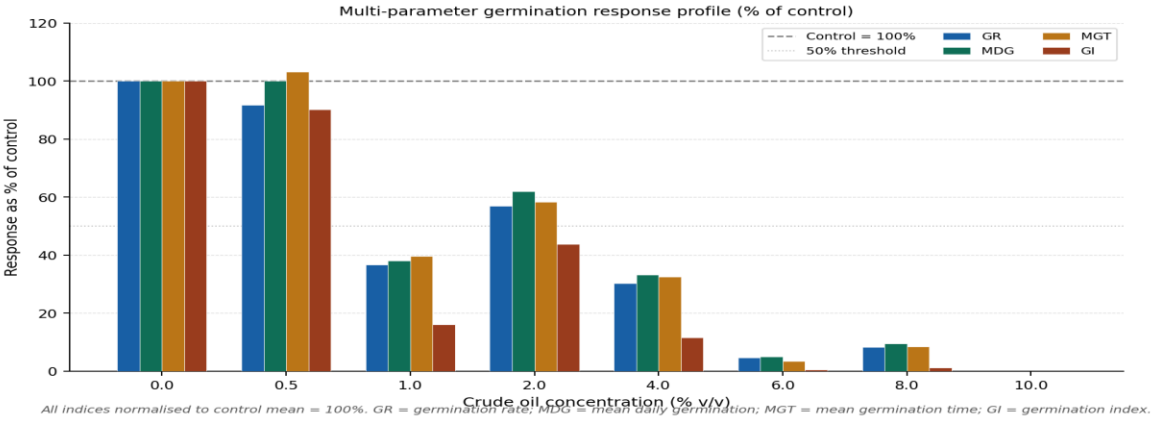

Figure B (Suggested). Normalised germination response profile showing GR, MDG, MGT, and GI expressed as percentages of respective control means (control = 100%) across all crude oil concentrations. Dashed line at 100% = control performance; dotted line at 50% = substantial impairment

threshold. All four indices collapse in parallel above 1.0% v/v. GR = germination rate; MDG = mean daily germination; MGT = mean germination time; GI = germination index.

Normalised values at 4.0% v/v: GR: 30.3% of control; MDG: 33.3%; MGT: 32.5%; GI: 11.6%. All indices reach 0% at 10.0% v/v.

4.2 Seedling Development

4.2.1 Seedling Length, Fresh Weight, and Dry Weight

Seedling length and fresh weight declined progressively with increasing crude oil concentration (Table 3; Figure C). Both parameters differed significantly among treatments (seedling length: $F = 20.35$, $P < 0.001$; fresh weight: $F = 13.67$, $P < 0.001$). Tukey's comparisons identified significant reductions relative to the control at concentrations >= 1.0% v/v. At 6.0% and 8.0% v/v, mean seedling lengths fell to 1.00 and 1.80 cm respectively, compared with 23.5 cm in the control (reductions of 95.7% and 92.3%). At 10.0% v/v, no seedling growth was recorded.

Dry weight did not differ significantly across treatments ($P > 0.05$), consistent with the literature indicating that this parameter is relatively insensitive to PHC stress at the biomass level (Masakorala et al., 2013).

4.2.2 Relative Water Content

RWC% remained stable at approximately 94-97% across concentrations of 0.0-4.0% v/v (Table 3; Figure 4). A significant reduction was detected only at 10.0% v/v ($F = 5.54$, $P < 0.01$), where RWC% collapsed to 0.0% in association with complete germination failure. At 6.0% and 8.0% v/v, mean RWC% values of 33.3% were recorded but were not statistically distinguishable from the control ($P > 0.05$) due to high variability from near-complete germination inhibition.

Trt (%)	Length (cm)	Fresh wt (g)	Dry wt (g)	RWC (%)
0.0	23.5a +/- 0.32	0.032a +/- 0.004	0.001 +/- 0.0002	97.2a +/- 0.30
0.5	22.8a +/- 1.80	0.030a +/- 0.004	0.001 +/- 0.0003	95.3a +/- 0.96
1.0	10.3bc +/- 1.40	0.011bc +/- 0.001	0.001 +/- 0.0001	94.6a +/- 1.1
2.0	14.3ac +/- 4.80	0.020ac +/- 0.007	0.001 +/- 0.0003	95.6a +/- 1.2
4.0	7.33bc +/- 2.20	0.010bc +/- 0.003	0.001 +/- 0.0004	94.9a +/- 2.1
6.0	1.00b +/- 1.00	0.002b +/- 0.002	0.0001 +/- 0.0003	33.3ab +/- 33.3
8.0	1.80b +/- 1.00	0.003b +/- 0.001	0.0013 +/- 0.0013	33.3ab +/- 33.3
10.0	0.00b +/- 0.00	0.000b +/- 0.000	0.000 +/- 0.000	0.000b +/- 0.000

Table 3. Effect of light crude oil dilutions on seedling length (cm), fresh weight (g), dry weight (g), and relative water content (RWC%) of Amaranthus hybridus L. seedlings (mean +/- SEM; n = 6). + = not significant; ** = $P < 0.01$; *** = $P < 0.001$. Different letters = significant differences (Tukey's HSD).

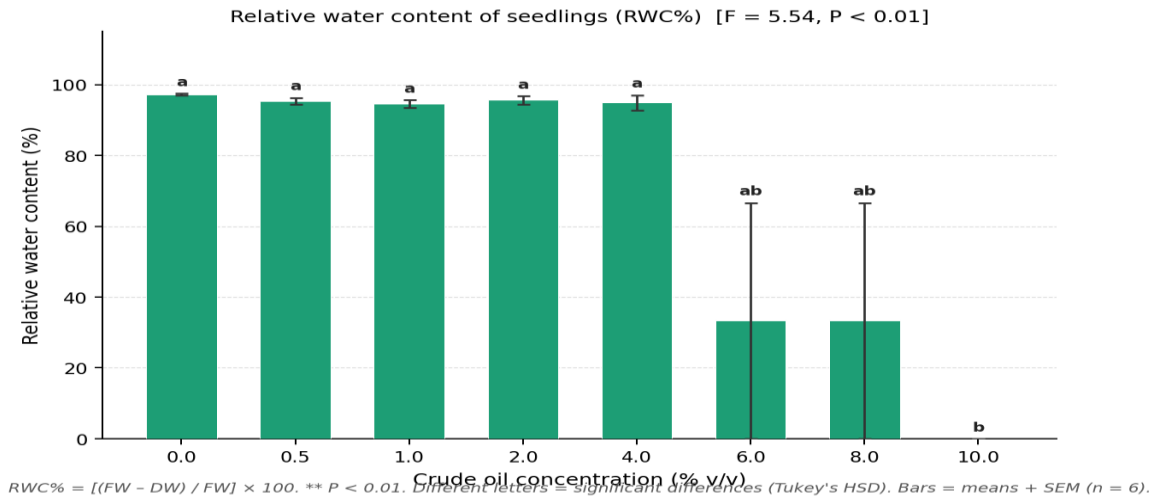

Figure 4. Effect of light crude oil dilutions on relative water content (RWC%) of Amaranthus hybridus L. seedlings. RWC% = [(FW - DW) / FW] x 100. Bars = means + SEM (n = 6). ** = P < 0.01 (F = 5.54). Different letters = significant differences (Tukey's HSD). Significant reduction vs. control only at 10.0% v/v.

RWC% maintained at ~94-97% for 0.0-4.0% v/v, indicating seedling water relations are preserved under moderate contamination despite severe germination inhibition.

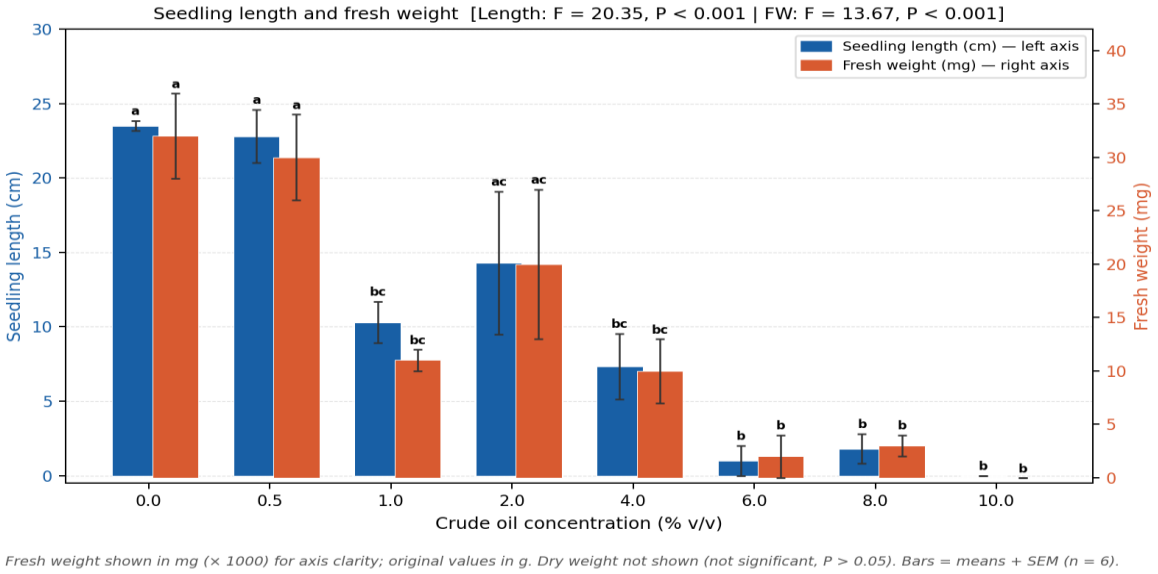

Figure C (Suggested). Seedling length (cm, left axis, blue bars) and fresh weight (mg = g x 1000, right axis, coral bars) of Amaranthus hybridus L. seedlings across light crude oil concentrations on dual axes for direct proportional comparison. Seedling length: F = 20.35, P < 0.001; Fresh weight: F = 13.67, P < 0.001. Dry weight not shown (not significant). Bars = means + SEM (n = 6). Different letters = significant differences (Tukey's HSD).

Both parameters show statistically identical concentration-response patterns, indicating unified growth suppression rather than independent effects. Reductions >= 95% relative to control at 6.0-8.0% v/v.

4.2.3 Seedling Emergence, Vigor, and Tolerance Indices

SE%, SVI, and TI all declined substantially with increasing crude oil concentration (Table 4; Figures 5, 6, and 7). Significant treatment effects were observed for all three parameters (SE%: F = 19.18, P < 0.001; SVI: F = 13.14, P < 0.001; TI: F = 10.19, P < 0.001). At 1.0% v/v, SVI dropped from 1646 (control) to 285, a reduction of 82.7% the steepest single-concentration decline observed across any parameter in this study. No seedling parameter values were obtainable at 10.0% v/v due to complete germination inhibition. TI at 0.5% v/v (0.97) confirmed this concentration was effectively subthreshold for A. hybridus.

Trt (%)	SE (%)	SVI	TI
0.0	76.7a +/- 3.33	1646a +/- 126	1.00a +/- 0.00
0.5	70.0ac +/- 5.80	1619a +/- 254	0.97a +/- 0.08
1.0	26.7b +/- 3.33	285b +/- 67.6	0.44ab +/- 0.06
2.0	43.3bc +/- 14.5	758bc +/- 429	0.60ab +/- 0.196
4.0	23.3bc +/- 8.80	343b +/- 153	0.60ab +/- 0.27
6.0	3.33bcd +/- 3.33	10.0b +/- 10.0	0.040b +/- 0.04
8.0	6.70bcd +/- 3.33	18.3b +/- 10.0	0.080b +/- 0.04
10.0	0.00bcd +/- 0.00	0.00b +/- 0.00	0.000b +/- 0.00

Table 4. Effect of light crude oil dilutions on seedling emergence percentage (SE%), seedling vigor index (SVI), and tolerance index (TI) of Amaranthus hybridus L. seedlings (mean +/- SEM; n = 6). *** = P < 0.001. Different letters = significant differences (Tukey's HSD). No values calculable at 10.0% v/v.

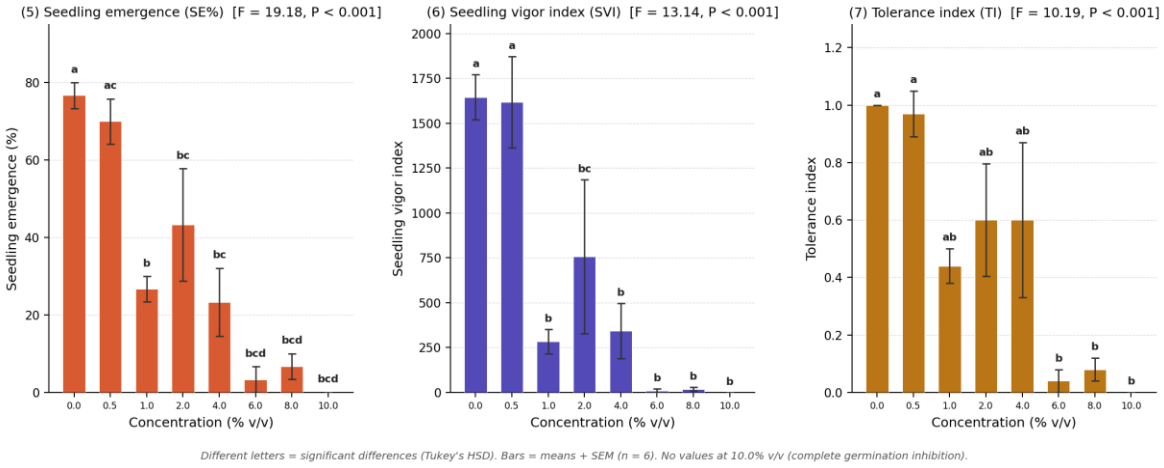

Figure 5. Effect of light crude oil dilutions on (5) seedling emergence percentage (SE%, F = 19.18, P < 0.001), Figure 6. Seedling vigor index (SVI, F = 13.14, P < 0.001), and (7) tolerance index (TI, F = 10.19, P < 0.001) of Amaranthus hybridus L. seedlings. Bars = means + SEM (n = 6).

Figure 7. Different letters = significant differences (Tukey's HSD). No values at 10.0% v/v (complete germination inhibition).

SE%: 0.0%: 76.7a; 1.0%: 26.7b; 4.0%: 23.3bc; 10.0%: 0.00bcd. | SVI: 0.0%: 1646a; 1.0%: 285b (82.7% reduction); 10.0%: 0.00b. | TI: 0.5%: 0.97 (subthreshold confirmed); TI < 0.50 at all concentrations >= 1.0% v/v.

Statistical note for all figures: Data are group means +/- SEM (n = 6 replicates per treatment). One-way ANOVA performed using SPSS v.26. Tukey's HSD post-hoc test applied where overall effects were significant. Significance levels: + = P >= 0.05 (not significant); ** = P < 0.01; *** = P < 0.001. Groups sharing the same letter code above bars are not significantly different. No calculated values were obtained for seedling parameters at 10.0% v/v because germination was completely inhibited at this concentration.

Discussion

The results demonstrate clear, concentration-dependent phytotoxic effects of light crude oil on the germination and early seedling development of Amaranthus hybridus L. across all measured parameters. The complete inhibition of germination at 10.0% v/v, and significant suppression evident above 1.0% v/v, are consistent with the mechanistic framework outlined by (Haider et al., 2021) and (Luo et al., 2024). Two principal pathways appear to operate simultaneously in this bioassay. First, hydrophobic PHC fractions restrict water availability for seed imbibition, depressing GR, MDG, MGT, GI, and CGV (Mekonnen et al., 2024). Second, direct chemical toxicity mediated by low-molecular-weight aromatic compounds in light crude oil including BTEX induces oxidative damage and membrane disruption in germinating embryos (Luo et al., 2024), explaining the abrupt transition to complete inhibition above 6.0% v/v.

The differential sensitivity of germination-stage versus growth-stage responses whereby dry weight was unaffected even as germination was progressively abolished corroborates a pattern reported broadly in the phytotoxicity literature (Masakorala et al., 2013; Almaroai & Eissa, 2025). The present data confirm that seedling length and fresh weight are the most responsive post-germination endpoints in A. hybridus under light crude oil stress, consistent with findings by (Odjegba & Sadiq, 2002) for spent engine oil and by Quinones-Aquilar et al., 2003, for maize in crude oil-contaminated soils.

The significant reduction in RWC% only at the highest concentration (10.0% v/v), while germination is substantially suppressed from 1.0% v/v upward, indicates that the primary bottleneck for A. hybridus crop establishment in moderately contaminated soils is germination failure rather than seedling desiccation. This distinction is practically important: remediation strategies targeting water availability alone would be insufficient at concentrations >= 1.0% v/v, where direct chemical toxicity appears to dominate (Mekonnen et al., 2024; Luo et al., 2024).

The pronounced declines in SVI and TI confirm that even 1.0% v/v substantially compromises the agronomic performance of A. hybridus. SVI at 1.0% v/v was only 17.3% of the control value (285 vs. 1646) the steepest single-step collapse observed. The near-linear SVI-concentration decline is consistent with dose-response patterns reported by (Agbogidi et al., 2007) for Zea mays and by (Almaroai & Eissa, 2025, Mansour etal,.2021) for Salicornia sinus-persica under graded PHC concentrations.

The high sensitivity of A. hybridus to light crude oil across the full measured concentration range

combined with its practical advantages of rapid germination, small seed size, ecological ubiquity in contaminated landscapes, dietary significance, and reproducible concentration-response relationships makes it a strong candidate for standardised phytotoxicity testing protocols. The phytotoxic threshold for A. hybridus germination, between 0.5% and 1.0% v/v light crude oil, is notably lower than EC50 values reported for maize (3.04%) and wheat (2.86%) in comparable TPH bioassays, confirming the species' relative sensitivity. Plants demonstrating reproducible concentration-response relationships are precisely the type of bioindicators recommended for short-term phytotoxicity screening (Masakorala et al., 2013; Luo et al., 2024).

Principal limitations of this study should be acknowledged. The bioassay was conducted under controlled laboratory conditions, and field soils introduce additional variables including texture, organic matter content, microbial activity, and hydrocarbon weathering that may modulate phytotoxicity. This study examined a single accession of A. hybridus; intraspecific variation in petroleum tolerance warrants future investigation. Future work should extend the bioassay to soil-based conditions, evaluate seed priming or rhizobacterial inoculation for restoring germination capacity under sublethal PHC concentrations, and assess PHC residue accumulation in harvestable biomass a food safety question directly relevant to contaminated agricultural landscapes.

6. Conclusion

This study demonstrates that light crude oil exerts significant, concentration-dependent phytotoxic effects on Amaranthus hybridus L. across all measured germination and seedling development parameters. Germination was completely inhibited at 10.0% v/v, with significant reductions in GP%, GR, MDG, MGT, GI, CGV, SVI, TI, seedling length, and fresh weight evident above 1.0% v/v. Dry weight was not significantly affected, confirming that germination-stage processes are the primary site of PHC phytotoxicity in A. hybridus. These findings establish A. hybridus as a sensitive, dose-responsive bioindicator for light crude oil contamination in agricultural soils, with implications for soil quality monitoring, crop risk management in oil-producing regions, and the design of standardised phytotoxicity screening protocols.

Figure Index

All figures embedded in this document were generated from the data reported in this manuscript using Python/matplotlib. Figures marked '(Suggested)' are additional visualizations recommended for inclusion in the published manuscript to complement the original figures.

Figure	Title	Section	Statistical test	Key finding
Fig. 1 (A,B)	Daily germination % Days 4 & 7	4.1.1	F=12.48/16.42, P<0.001	Suppression above 1.0% v/v; zero at 10.0%
Fig. A (Sugg.)	Germination time course Days 1-7	4.1.1	(descriptive)	2.0% v/v shows progressive trajectory; >=6.0% flat
Fig. 2	Inhibition of final germination (IFG%)	4.1.2	F=19.18, P<0.001	100% IFG at 10.0% v/v
Fig. 3	Coefficient of germination velocity (CGV)	4.1.3	F=14.27, P<0.001	Near-zero at >=6.0% v/v

Fig. B (Sugg.)	Multi-parameter response (% of control)	4.1.3	(normalised)	All 4 indices collapse in parallel >1.0%
Fig. 4	Relative water content (RWC%)	4.2.2	F=5.54, P<0.01	Significant only at 10.0% v/v
Fig. C (Sugg.)	Seedling length & fresh weight (dual-axis)	4.2.1	F=20.35/13.67, P<0.001	Identical proportional decline; dry wt unchanged
Figs. 5,6,7	SE%, SVI, and TI (3-panel)	4.2.3	F=19.18/13.14/10.19, P<0.001	SVI drops 82.7% at 1.0% v/v; TI<0.50 at >=1.0%

Figure index. Original figures (1-5) and suggested additional figures (A-C). All produced from data reported in this manuscript using Python/matplotlib v3.10.

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b) Aiad Abdelkareim Akhreim Alzway: He likely oversaw the surface sterilization protocols using formaldehyde and ensured the maintenance of sterile conditions during the incubation process.

c) Ghazala Ahmad Hamaden Mansour: He has involved the botanical classification of Amaranthus hybridus L. and the detailed quantification of seedling development parameters such as seedling length and fresh weight.

d) Idress Hamad Attitalla: As the corresponding author and a senior member he has provided overall project supervision, conceptualized the research objectives, and performed the formal statistical analysis (One-way ANOVA and Tukey's HSD) using SPSS v.26.

Ethical Approval: Not applicable. This study was a laboratory-based botanical assessment using seeds of Amaranthus hybridus L. and did not involve any human or animal subjects.

Consent to Participate: Not applicable. This research did not involve human participants.

Consent to Publish: All authors have reviewed the manuscript and provided their consent for its publication.

Competing Interests: The authors declare that they have no competing interests.

Data Availability Statement: The data supporting the findings of this study are available within the article's tables and figures, including detailed germination indices and seedling development parameters. Specifically, all primary statistical results and concentration-response data are presented in Tables and Figures.