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# Assay dependence of concentration responses to Terminalia leaf and bark extracts in four food crops

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**Research Article**

**Keywords:** allelopathy, Terminalia, seedling growth, extract concentration, assay context, uncertainty

**Posted Date:** November 11th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-11264654/v1

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**Additional Declarations:** No competing interests reported.

# Abstract

Early-growth bioassays are widely used to screen tree–crop interactions, but their interpretation depends on exposure, endpoint and assay context. We analysed replicate records for leaf and bark extracts of *Terminalia bellirica* and *Terminalia chebula* applied to four food crops at 2, 5 and 10% mass per volume after 24, 48 or 72 h extraction. The dataset contained 864 treated records and 96 controls, with four complete outcomes. Contrasts among extract treatments avoided uncertain control sharing, while heterogeneous-variance intervals, multiplicity adjustment and exact single-record deletion assessed uncertainty and influence. Increasing concentration from 2 to 10% reduced laboratory root length by 46.14% for *T. bellirica* and 48.87% for *T. chebula*, compared with 3.24% and 15.94% in pots. Pot *T. chebula* germination declined by 11.39 percentage points on the nominal seed denominator while dry mass increased by 11.86%. Aggregate pot shoot-length increases did not survive multiplicity adjustment. Extending extraction from 24 to 72 h reduced pot germination by 6.00 and 6.89 percentage points, respectively. These results identify endpoint discordance and assay-dependent concentration responses rather than a universal crop-tolerance ranking. Laboratory and pot endpoints differed in age and medium, and inference remains conditional on the independence of the source replicate records. The analysis provides a reproducible basis for prioritising matched-exposure follow-up, without establishing chemical causation or field compatibility.


# Introduction

The value of an agroforestry tree depends partly on how its associated crops respond to the chemical and physical environment that the tree creates. Trees can provide products and ecosystem services, but these benefits do not establish the suitability of every tree–crop combination (Jose 2009). Laboratory assays of plant extracts offer a tractable way to identify responses that deserve further investigation. Their relevance to management depends on a second question: whether the response being measured remains informative when the assay conditions change.

This distinction is especially relevant to *Terminalia chebula* Retz. and *Terminalia bellirica* (Gaertn.) Roxb. Both species have already been examined for effects of leaf and bark extracts on crop germination and early growth. Thapaliyal et al. (2008) therefore provide direct precedent for the donor species and plant materials investigated here. Regional studies of agroforestry trees also show that recipient crops and plant materials can respond differently within a common screening protocol (Bhatt and Todaria 1990; Pant et al. 2018; Vasishth et al. 2020; Naik et al. 2021).

Several processes can separate laboratory and pot responses. The concentration applied to filter paper is not equivalent to the concentration reaching roots in a soil-containing medium. Sorption, microbial transformation and the composition of the soil solution can alter exposure, while water and nutrient conditions affect the recipient plant's response (Inderjit and Weston 2000; Inderjit 2005; Scavo et al. 2019). Root exudates and decomposing plant materials also differ from an experimentally prepared aqueous extract (Bertin et al. 2003). Phenolic compounds are plausible participants in plant–soil

interactions, but their occurrence in plants cannot establish which constituents cause a response in an uncharacterised extract (Li et al. 2010). Concentration and extraction duration provide complementary information. Increasing the mass of plant material per unit volume changes the nominal exposure. Increasing the time that the material remains in water may change both the amount and composition of the dissolved mixture. These operations are not interchangeable, and the duration used to prepare an extract is not a repeated observation time for a seedling. Mechanistic reviews identify multiple pathways through which allelochemicals may affect germination and growth, reinforcing the need to retain different response variables toxicity (Inderjit and Duke 2003; Cheng and Cheng 2015).

Germination is an early component of establishment, whereas root or shoot elongation describes growth among the seedlings that were measured. Dry mass adds a further dimension and need not track elongation. A mean score that gives equal weight to all endpoints can hide disagreement and double-count correlated responses. Dependence introduced by a common control is a recognised issue in ecological effect-size analysis (Hedges et al. 1999; Lajeunesse 2011, 2015), and the statistical behaviour of control-relative response indices has long been recognised as consequential in allelopathy assessment (Williamson and Richardson 1988).

Here, we reanalyse a factorial dataset for two *Terminalia* species, leaf and bark extracts, four food crops, three nominal concentrations and three extraction durations in laboratory and nursery-pot assays. We ask whether increasing concentration from 2 to 10% produces a consistent change within an assay, whether that concentration response differs between laboratory and pot conditions, and whether increasing extraction duration from 24 to 72 h changes the result. We also examine whether root and shoot responses disagree without presuming that similarly labelled records establish seedling-level pairing.

## Materials and methods

# Study setting and plant materials

The experiments were conducted at the College of Forestry, Ranichauri, Uttarakhand, India, during the August 2023–June 2024 study period. Experimental procedures are documented in Deepali Dobhal's 2024 MSc thesis, An assessment of Phytotoxic Potential of *Terminalia chebula* and *Terminalia bellirica* on germination and growth pattern of seasonal food crops of Garhwal Himalaya.

The donor materials were leaves and bark of *T. chebula* collected at Nagni Sera and *T. bellirica* collected at Bahera, near Chamba in Tehri Garhwal. The tree and parts were identified and subsequently verified by Dr. C. S. Dhanai and Dr Bhupendra Singh, both Assistant Professor, Department of Silviculture and Agroforestry, College of Forestry, Ranichauri, VCSG Uttarakhand University of Horticulture and Forestry. Material was shade-dried for approximately one week and ground mechanically. Separate aqueous preparations contained 2, 5 or 10 g of dry material per 100 mL of distilled water, equivalent to 2, 5 and

10% mass per volume. For each concentration, material was soaked separately for 24, 48 or 72 h at room temperature. Extracts were filtered through muslin followed by Whatman No. 1 filter paper.

The four recipient crops were foxtail millet (*Setaria italica*; cultivar not recorded), barley (*Hordeum vulgare*, PRB 701), ricebean (*Vigna umbellata*, PRR-1) and lentil (*Lens culinaris*, BL-126). The working correspondence C1, C2, C3 and C4, respectively, is inferred from the order of crops in the thesis and was also explicitly labelled as such in the workbook. Accordingly, crop codes are retained in the results and figures.

## Laboratory and pot assays

The laboratory protocol specifies three replicates of 25 seeds per treatment in Petri dishes with Whatman No. 1 filter paper moistened with extract, at approximately 25 ± 1°C. Distilled water was used for the control series, and extract was added as required to maintain moisture. Germinated seeds were counted through day 7. The procedural text describes length measurement at seven days after germination and 15 sampled seedlings across three replicates. Radicle and plumule lengths were measured in centimeters. Seedlings were oven-dried at 73°C for 24 h and dry mass was recorded in grams.

The nursery protocol describes three replicates with 75 seeds per treatment in pots containing sand, soil and farmyard manure in a 1:2:1 ratio. Pots received the assigned extracts during watering. Germination was followed during the 21-day experiment; five seedlings per replicate were sampled for root and shoot length at its conclusion, and dry mass was measured after the same drying procedure. The pot experiment used completely randomized design.

For cross-assay presentation, radicle/root length is termed root length and plumule/shoot length is termed shoot length; the original labels remain in the source data. This is a comparison of homologous response domains, not an assumption of identical developmental stages. Laboratory and pot measurements differ in medium, growth conditions and measurement timing. Their difference is therefore an assay-context contrast.

## Data provenance and analytical units

A workbook was maintained through-out the experimental duration and later transferred systematically to excel worksheets. Each source R value is treated computationally as one recorded replicate summary, not as an individual seed or seedling. The individual seedlings sampled within dishes or pots do not increase the replication used for inference. This distinction is essential to avoid pseudoreplication (Hurlbert 1984). Constructed identifiers join source records for processing but do not prove physical dish, pot, seedling or extract-batch identity. The interpretation of standard errors is conditional on R1–R3 representing independent experimental units within a treatment cell.

## Estimands and uncertainty

The primary concentration contrast compares the observed 10% and 2% preparations within donor species, plant part, crop and assay. Positive length and mass measurements are analysed on their natural-log scale. This makes the back-transformed effect a ratio of geometric means of recorded replicate summaries. It is not a logarithm of an arithmetic treatment mean, and it is not interchangeable with every response ratio used in meta-analysis (Hedges et al. 1999; Feng et al. 2014). Recorded integer germination counts were converted to nominal percentages as 100 times the count divided by 25, using the workbook's nominal seeds-sown field. The pot denominator is supported by the procedural total of 75 seeds across three replicates rather than by a separate observed sown-count register. Germination contrasts are reported in percentage points on this nominal basis. No rounded percentage was used to reconstruct a seed count, and no binomial model assumes independent responses of the seeds within a dish or pot.

For a specified contrast, the estimate is a weighted sum of transformed cell means. Concentration contrasts average equally across the three extraction durations; extraction-duration contrasts compare 72 with 24 h and average equally across the three concentrations. Equal weights define a finite experimental-grid estimand and are applied across treatment conditions within one endpoint. They do not combine correlated endpoints into a composite score. The 5% concentration and 48 h duration contribute to descriptive response profiles and the appropriate marginal contrasts.

For independent source cells, the estimated variance is the sum of squared contrast weights multiplied by each cell's sample variance divided by its observed replication. Approximate t confidence intervals use Welch–Satterthwaite degrees of freedom (Satterthwaite 1946; Welch 1947). A ratio below one indicates a smaller response at the higher concentration or longer extraction duration; a ratio above one indicates a larger response. The assay-context effect subtracts the laboratory log contrast from the pot log contrast, so its exponential is a ratio of concentration-response ratios. For germination the corresponding context effect is a difference of percentage-point differences.

Root and shoot contrasts are reported separately. To describe their difference without assuming verified cross-trait pairing, each cell's variance bound is calculated by squaring the sum of the marginal standard deviations and dividing by its replication. These bounds are combined using squared contrast weights. This Cauchy–Schwarz calculation supplies an upper bound on variance for specified population variances. The resulting interval is therefore an approximate sensitivity envelope. Root–shoot inference is not based on its P values. For the individual endpoints, familywise multiplicity control follows Holm (1979) within the explicitly defined contrast families reported in the thesis Deepali 2024. Nominal 95% confidence intervals accompany effect estimates; these intervals are not simultaneous familywise confidence intervals. Statistical thresholds are used as summaries of evidence rather than as substitutes for effect magnitude, uncertainty and biological interpretation (Wasserstein and Lazar 2016).

## Sensitivity analysis and reproducibility

Sensitivity analyses examine the influence of deleting one observed R value at a time and the consequences of alternative handling of the recorded R labels. The exact single-record deletion range is an influence diagnostic, not a confidence interval and not external validation. No large bootstrap sample is presented as though it created biological replication. Arithmetic cell summaries and control-relative responses are retained as descriptive outputs, but ambiguous control identity is not allowed to determine the primary concentration and duration contrasts.

All transformations, contrast weights, multiplicity families, software versions and output tables are documented in the accompanying executable analysis. No values were imputed or excluded. Figures are generated from these outputs. ChatGPT was used to assist analytical planning, code generation, literature checking and manuscript drafting to a limited extent. The executed code and its results are supplied for scrutiny; responsibility for scientific interpretation and the submitted version remains with the named authors.

# Results

The source excel worksheet comprised 960 replicate-labelled rows: 864 extract-treated records in 288 factorial cells and 96 controls in 32 labelled sets (Table 1). All four outcomes were present in every row, giving 3,840 measurements. Two complete control vectors, each comprising three R records and four traits, were identical across donor datasets for the pot bark C3 and C4 series. This numerical duplication does not establish physical reuse, but reinforces the decision to keep controls out of the primary contrasts.

Table 1
Experimental grid and analysed records

| Component | Recorded structure |
|---|---|
| Donors | *T. bellirica* and *T. chebula* |
| Assays | Laboratory and nursery pots; different endpoints |
| Donor organs | Leaf and bark |
| Crop codes | C1–C4; four crops, name mapping provisional |
| Concentrations | 2, 5 and 10% mass per volume |
| Extraction durations | 24, 48 and 72 h; separate preparation treatments |
| Treated grid | 288 cells × 3 source R records = 864 rows |
| Controls | 32 labelled sets × 3 source R records = 96 rows |
| Outcomes | Germination count; shoot length (cm); root length (cm); dry mass (g) |
| Data completeness | 960 rows × 4 outcomes = 3,840 values; no missing outcomes |

R labels are source replicate summaries. The primary analysis uses treated cells; seedlings sampled within a dish or pot are not additional replicates.

## Concentration responses depend on assay context

Increasing concentration from 2 to 10% reduced all four aggregate laboratory responses for both donors after adjustment across the 32 primary tests (Fig. 1). For *T. bellirica*, geometric mean shoot length decreased by 28.04% (nominal 95% confidence interval, 25.14–30.82% decrease), root length by 46.14% (42.77–49.31%) and dry mass by 20.00% (15.34–24.41%). The corresponding decreases for *T. chebula* were 40.09% (34.87–44.89%), 48.87% (43.77–53.50%) and 31.58% (26.25–36.53%). Germination declined by 5.44 percentage points for *T. bellirica* and 11.11 points for *T. chebula*, on the nominal 25-seed basis.

Pot responses were more differentiated. *T. chebula* retained a root-length decrease of 15.94% (12.48–19.26%) and a germination decrease of 11.39 percentage points (8.52–14.25 points), whereas dry mass increased by 11.86% (4.58–19.64%; Holm-adjusted P = 0.025). Thus, a positive mass response co-occurred with adverse establishment and root-length responses in the finite-grid averages. Pot shoot-length point estimates were positive for both donors, but neither was significant after the primary Holm correction: +3.43% for *T. bellirica* (adjusted P = 0.070) and + 5.64% for *T. chebula* (adjusted P = 0.056). The *T. bellirica* pot concentration effects on germination, root length and mass also did not pass that correction. These results should not be described as demonstrated stimulation of pot shoot growth or as absence of a biological effect.

The formal context comparisons corroborated differences between assay-specific concentration responses for all three growth variables in both donors (Table 2). The pot-to-laboratory ratio of concentration-response ratios was 1.797 for *T. bellirica* root length and 1.644 for *T. chebula* root length. For shoot length it was 1.437 and 1.763, respectively. These quantities compare proportional concentration effects within each assay; they do not mean that pot seedlings were 79.7% or 64.4% longer than laboratory seedlings. The concentration effect on germination was less clearly modified by context: the pot-minus-laboratory differences were + 3.56 percentage points for *T. bellirica* (adjusted P = 0.079) and − 0.28 points for *T. chebula* (adjusted P = 1.000).

Table 2
Modification of the concentration response between assay contexts

| Donor | Outcome | Context effect | Nominal 95% interval | Holm P |
|---|---|---|---|---|
| *T. bellirica* | Germination | + 3.56 pp | + 0.83 to + 6.28 | 0.079 |
| *T. bellirica* | Shoot length | 1.437 | 1.374 to 1.504 | < 0.001 |
| *T. bellirica* | Root length | 1.797 | 1.683 to 1.918 | < 0.001 |
| *T. bellirica* | Dry mass | 1.214 | 1.130 to 1.305 | < 0.001 |
| *T. chebula* | Germination | -0.28 pp | -3.68 to + 3.13 | 1.000 |
| *T. chebula* | Shoot length | 1.763 | 1.612 to 1.928 | < 0.001 |
| *T. chebula* | Root length | 1.644 | 1.484 to 1.821 | < 0.001 |
| *T. chebula* | Dry mass | 1.635 | 1.481 to 1.805 | < 0.001 |

For lengths and mass, the context effect is the pot 10:2% response ratio divided by the laboratory 10:2% ratio (null = 1). For germination, it is the pot-minus-laboratory difference in nominal percentage-point changes (pp; null = 0). P values belong to the secondary family of 16 aggregate concentration and duration context tests. The assays differ in age as well as medium.

## Crop and organ strata reveal heterogeneous directions

All 16 donor-by-organ-by-crop combinations had negative laboratory shoot-length concentration contrasts, whereas ten had positive pot point estimates (Fig. 2). For root length, 15 combinations had negative laboratory estimates and ten had negative pot estimates; five changed from negative in the laboratory to positive in pots. These are sign descriptions of observed contrasts. They show why the donor-level averages must be read together with the crop and organ strata.

Intermediate-dose comparisons also showed that a single monotonic curve would not describe every sample profile. Among the 16 donor–organ–crop combinations, the 2-to-5% and 5-to-10% root-length steps had opposite signs in 12 pot profiles and five laboratory profiles. The corresponding counts for shoot length were eight and three. For dry mass they were ten and seven. These patterns concern the means at the three tested concentrations, averaged over extraction duration. They neither locate an optimum nor establish hormesis, because concentrations below 2%, a verified common zero-dose reference and a sufficiently resolved response curve are absent from the primary comparison. Full step contrasts are supplied in Online Resource 2.

# Extraction duration has a distinct response pattern

Extending extraction from 24 to 72 h reduced pot germination by 6.00 percentage points for *T. bellirica* and 6.89 points for *T. chebula*; both effects remained supported after the primary Holm correction

(Fig. 3). Their laboratory germination changes were smaller, − 1.50 and − 1.61 points, respectively, and were not supported after correction. The secondary context comparisons indicated more negative duration effects in pots by 4.50 points for *T. bellirica* (95% interval − 7.17 to − 1.83; adjusted P = 0.011) and 5.28 points for *T. chebula* (− 8.79 to − 1.76; adjusted P = 0.033).

For *T. bellirica*, longer extraction reduced laboratory shoot length by 7.60%, root length by 13.73% and dry mass by 11.36%; these effects survived correction. In pots, shoot length and mass decreased by 6.14% and 13.33%, respectively, whereas the root-length estimate was close to zero and uncertain. For *T. chebula*, no laboratory duration effect passed the primary correction. Its pot root-length decrease of 9.86% (95% interval 6.29–13.30% decrease) did, whereas the estimated 7.70% decrease in pot dry mass did not. Concentration and duration therefore produced different endpoint patterns and cannot be collapsed into a single concentration-times-duration exposure score.

# Relative root and shoot responses and influence sensitivity

The marginal root-to-shoot concentration-response ratio decreased for both donors in both assays (Fig. 4). The estimated change was − 25.16% for *T. bellirica* in the laboratory and − 6.45% in pots; the corresponding *T. chebula* estimates were − 14.65% and − 20.43%. The approximate covariance-bounded envelope for the *T. chebula* laboratory contrast included zero. These comparisons describe how the two marginal length responses change relative to one another. They do not measure biomass partitioning within a plant, and no claim of a physiological trade-off is inferred from them.

The main point estimates were not driven by a single source record (Table 3). Across all exact single-record deletions relevant to the *T. chebula* pot mass contrast, its increase ranged from 10.73 to 13.36%; the corresponding root-length decrease ranged from 15.47 to 16.39%. The *T. bellirica* laboratory root-length decrease remained between 45.49 and 46.90%. These ranges assess point-estimate influence only and do not establish the stability of every confidence interval or adjusted P value.

Table 3
Influence of individual records on selected concentration contrasts

| Donor and assay | Outcome | Full estimate (%) | Single-deletion range (%) |
|---|---|---|---|
| *T. bellirica* / laboratory | Root length | -46.14 | -46.90 to -45.49 |
| *T. chebula* / laboratory | Root length | -48.87 | -49.62 to -48.09 |
| *T. chebula* / pot | Root length | -15.94 | -16.39 to -15.47 |
| *T. chebula* / pot | Dry mass | + 11.86 | + 10.73 to + 13.36 |

Every full estimate uses all source observations. Each deletion omits one R record from one contributing cell and leaves all other cells unchanged. Ranges are point-estimate influence diagnostics.

Treating R labels as hypothetical blocks left the balanced-grid point estimates unchanged. Across pot sensitivity comparisons, the ratio of the R-block to the pooled completely randomised standard error

ranged from 0.917 to 1.015. Only one of 16 conventional unadjusted 0.05 decisions changed: the *T. bellirica* pot root-length concentration contrast had P = 0.0486 under the pooled completely randomised calculation and P = 0.0518 with the hypothetical R blocks. That contrast was already unsupported after the primary Welch–Holm analysis.

## Discussion

# What the cross-assay contrast adds

The clearest result is that the proportional effect of increasing extract concentration was strongly assay-dependent for seedling length and mass, while germination did not show the same general pattern. A laboratory screen alone would emphasize the negative concentration responses of both donors across all endpoints. The pot measurements instead retain adverse root and germination responses for *T. chebula* alongside an increase in dry mass among the recorded seedlings. This disagreement is directly relevant to how early-stage screens are interpreted: a less negative length response in one assay cannot justify calling an extract, tree or tree–crop combination broadly compatible.

The present study extends rather than displaces the earlier *Terminalia* screening literature. Thapaliyal et al. (2008) already tested the two donors and their leaf and bark extracts, and Isin Ozkan et al. (2019) provide an additional *Terminalia chebula* phytotoxicity study. The contribution here is the separation of concentration effects, extraction-duration effects and their modification across assay contexts using the corrected replicate-level records. It also makes explicit the uncertainty and the information that a control-dependent interpretation would require. Meta-analytic evidence supports context dependence in allelopathy research generally (Zhang et al. 2021), but that broader literature does not turn the current single-site preparations into replicated samples of the species' ecological behavior.

Studies designed to compare laboratory and field predictive performance are particularly useful precedents because they ask whether the screening endpoint carries information across conditions (Seal et al. 2008). Our pot assay is not a field validation of the Petri-dish assay: substrate, endpoint age and exposure management all differ, and no prespecified laboratory model was evaluated against an independent field dataset. The appropriate finding is assay dependence within this experiment. It identifies contrasts worth testing at matched ages and measured exposures.

The stronger negative concentration responses in the laboratory are consistent with several exposure-related explanations, including differences in sorption, transformation and root contact in a soil-containing medium. The soil literature shows why bioavailability and microbial activity can change the behavior of allelochemicals (Inderjit 2005; Scavo et al. 2019), while bioassay-design studies caution that extraction procedure, pH, nutrients and osmotic conditions can influence measured responses (Inderjit and Nilsen 2003). None of those pathways can be assigned a causal share of the present context contrast.

The distinct extraction-duration pattern is nevertheless informative. Longer extraction had a stronger adverse germination signature in pots than in the laboratory, even though concentration-associated growth reductions were generally greater in the laboratory. Current syntheses describe multiple physiological targets and interactions rather than one universal allelopathic mechanism (Choudhary et al. 2023; Kumar et al. 2024) and present study is compatible with the existing complexity reported in multiple studies.

Recent work with agroforestry leaf powders includes crop growth and yield endpoints (Abraha et al. 2025), whereas wheat grown under *T. chebula* trees has been evaluated through a field-oriented set of growth and yield measurements (Kumar et al. 2025). Such studies address exposure pathways and production outcomes that this short-term extract experiment wasn't able to accomodate. Advances in allelopathy-based weed management likewise depend on chemical characterization, delivery, crop selectivity and performance under realistic conditions (Macías et al. 2019).

# Endpoints should remain separate

The *T. chebula* pot results illustrate the cost of using a single response as a proxy for compatibility. The positive mass contrast and negative root-length and germination contrasts describe different properties of the observed seedlings and seed cohorts. Reduced establishment could change which seedlings survive to be measured, and the available mass records do not independently establish whether the value is per seedling or a pooled sample.

The root–shoot comparison makes discordance visible without imposing an unverified seedling pairing. Unknown cross-trait covariance widens the range of plausible uncertainty. Reporting that uncertainty is preferable to estimating a precise correlation from reconstructed R identifiers.

Similarly, opposite signs in the two observed concentration steps should not be promoted to a claim of beneficial low-dose stimulation. Dose-response shape can depend on the exposure range and assay conditions, and an endpoint contrast between 2 and 10% says nothing about whether 2% improves or impairs the response relative to water. The control-independent approach deliberately sacrifices that zero-dose interpretation to make its primary estimates less sensitive to unresolved control reuse. Williamson and Richardson (1988) examined indices comparing a treatment with its own independent control. The present high-versus-low exposure comparison answers a different question: it measures a relative concentration response without identifying either concentration as safe or optimal.

# Strength and limits of the statistical evidence

The analysis preserves the factorial treatment structure in its contrasts and reports crop–organ strata rather than pooling all observations into a single toxicity ranking. Equal weighting refers to the tested treatment grid, so the aggregate effects are properties of that grid. In particular, a comparison between donor averages cannot separate species effects from the particular collected material, donor sites or unrecorded extract batches. The underlying biological sampling limits persist even when the computational contrasts have narrow intervals.

The inferential framework also distinguishes uncertainty from influence. Heterogeneous cell variances enter the Welch approximation; Holm adjustment addresses specified test families; and exact single-record deletions show how individual observations affect point estimates. The limitations of three replicate summaries per cell are therefore stated alongside the effect estimate. P values near a threshold, including the pot shoot-length increases, are interpreted with the multiplicity adjustment and the interval definition in view (Wasserstein and Lazar 2016).

The next informative experiment would retain the donor–organ–crop structure while recording independent donor or preparation batches, randomized dish/pot identities and common measurement ages. It would quantify applied volume and chemical exposure, use explicitly identified controls and measure establishment together with survivor growth. A field phase would then need realistic litter or tree-mediated exposures and crop productivity outcomes. Regional screening work provides useful candidate materials (Bhatt and Todaria 1990; Naik et al. 2021), but the decision to advance a combination should be supported by agreement across relevant endpoints rather than by one positive growth response.

## Conclusions

In the supplied factorial dataset, increasing *Terminalia* extract concentration from 2 to 10% produced substantially more negative length responses at the laboratory endpoint than at the pot endpoint. Germination did not follow that general pattern, and *T. chebula* pot records combined lower germination and root length with higher dry mass. Extending extraction from 24 to 72 h produced a distinct adverse pot-germination response. These findings show why concentration, extraction duration and response endpoints should remain separate when interpreting early-stage tree–crop screens. The contribution is a reproducible analysis of assay-dependent responses with explicit uncertainty and influence checks. The data do not establish a safe concentration, a mechanism of allelopathy, a donor-species toxicity ranking or field crop compatibility. Confirmation of the original experimental-unit and measurement metadata remains necessary before the numerical evidence is used for species- or crop-specific recommendations.

## Declarations

**A. Acknowledgments**: The Authors acknowledge the lead of HoD Professor VP Khanduri of Silviculture and Agroforestry Department Dean, College of Forestry at that time Prof Arvind Bijalwan along with providing necessary staff and material support during the course of investigation.

**B. Author contributions:** Agreed the contributions of Deepali Dobhal, C. S. Dhanai and Ajaya Paliwal and Bhupendra Singh. We also confirm approval of the final manuscript. Proposed roles for review are: investigation and original study records by Deepali Dobhal; concept and supervision by C. S. Dhanai; technical inputs and reanalysis of the results were provided for manuscript preparation, Ajaya Paliwal and Bhupendra Singh.

**C. Clinical trial number**: The reported experiments concern plant materials and do not involve human participants or animals so clinical trial number not applicable.

**D. Consent to participate**: Consent to participate is not applicable.

**E. Consent to publish**: Not applicable. The manuscript does not contain any individual person's data or images that require consent for publication.

**F. Conflicts of interest**: There is no conflict of interest. This study was the sole work of Ms Deepali while the other members provided technical inputs and general guidelines for the scientific work.

**G. Ethical approval**: Plant guidelines: The leaves and bark of Terminalia chebula were collected from village forest land at Nagni Sera village, while the leaves and bark of Terminalia bellirica were collected from Bahera village near Chamba, Tehri Garhwal, Uttarakhand. Herbarium Details: No voucher specimen was deposited in a public herbarium at the time of the original study; therefore, herbarium deposition number is unavailable. Voucher specimens: The collected leaves and bark were sun-dried, ground into powder and stored in airtight containers during the experimental study. The original plant material is no longer available. Photographs taken during collection are available with the lead author. The plant materials were collected from village forest land with the verbal permission of the respective local villagers/ landowners. No separate written collection permit was obtained at the time of collection; no identifiable people are shown in the selected photographs.

**H. Data availability statements**: Original Data log book is in the possession of lead author Ms Deepali and data excel worksheet generated from the log book is in the possession of the corresponding author which is provided in the supplementary section.

**I. Funding:** The study was part of post-graduate research of MSc Forestry (SAF) student and was funded through departmental resources of Silviculture and Agro-forestry department, College of Forestry, VCSG Uttarakhand University of Horticulture and Forestry, Ranichauri-249199, Tehri Garhwal, Uttarakhand.

**F. Prior dissemination:** The experiments were documented in Deepali Dobhal's MSc thesis (2024). The present numerical analysis uses the original-record workbook, not the thesis's outcome tables and this manuscript is not under consideration elsewhere.

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# Figures

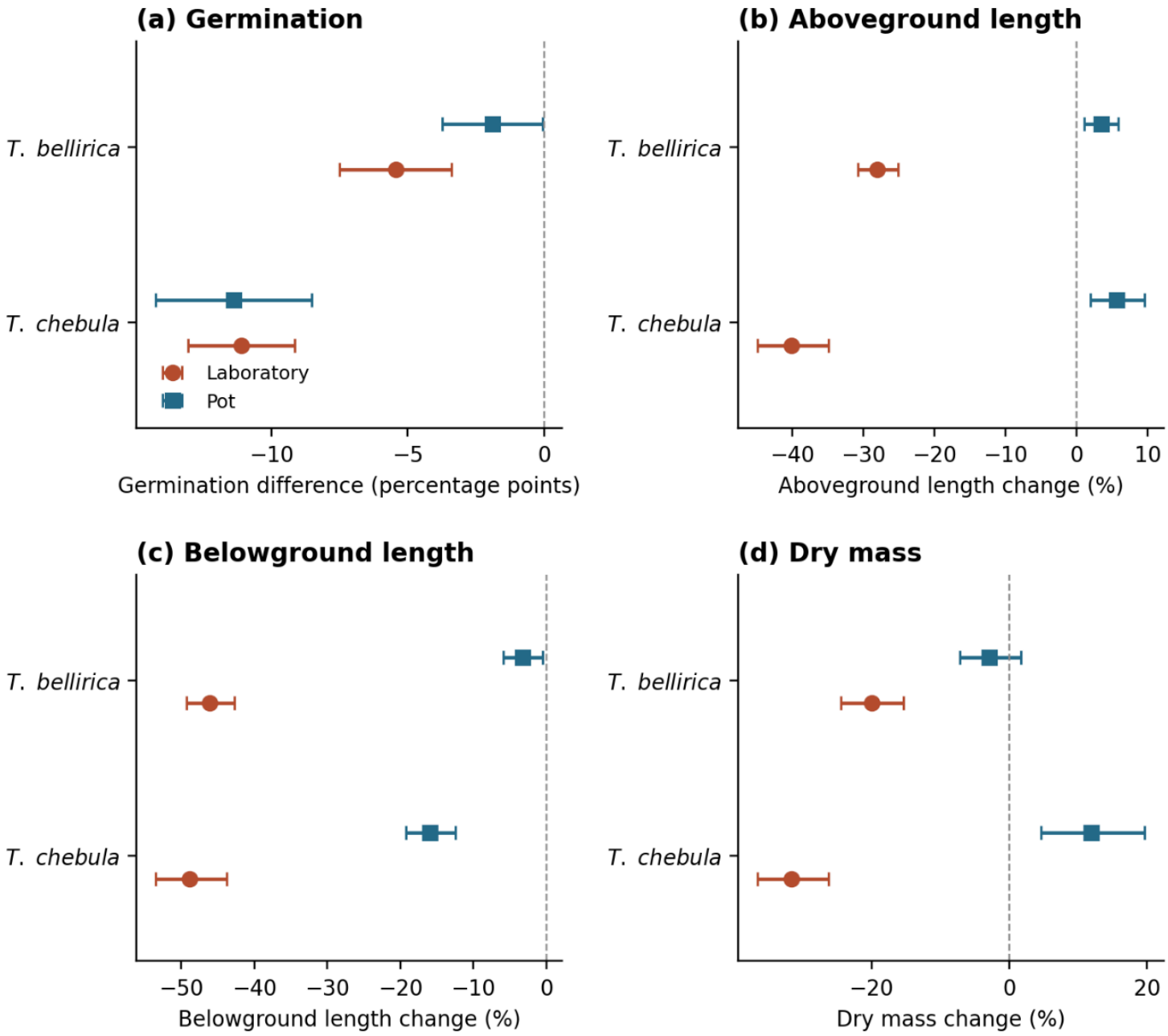



**Figure 1**

Concentration escalation from 2 to 10% mass per volume in laboratory and pot assays. Points average equally over the two donor organs, four crop codes and three extraction durations within each donor species. Bars are pointwise approximate 95% Welch intervals. Germination is a nominal percentage-point difference; lengths and mass are percentage changes in geometric means. Dashed lines indicate zero change. Laboratory circles and pot squares distinguish assay contexts. The positive pot shoot-length estimates did not survive the 32-test Holm adjustment. Different measurement ages prevent attribution of context differences to substrate alone

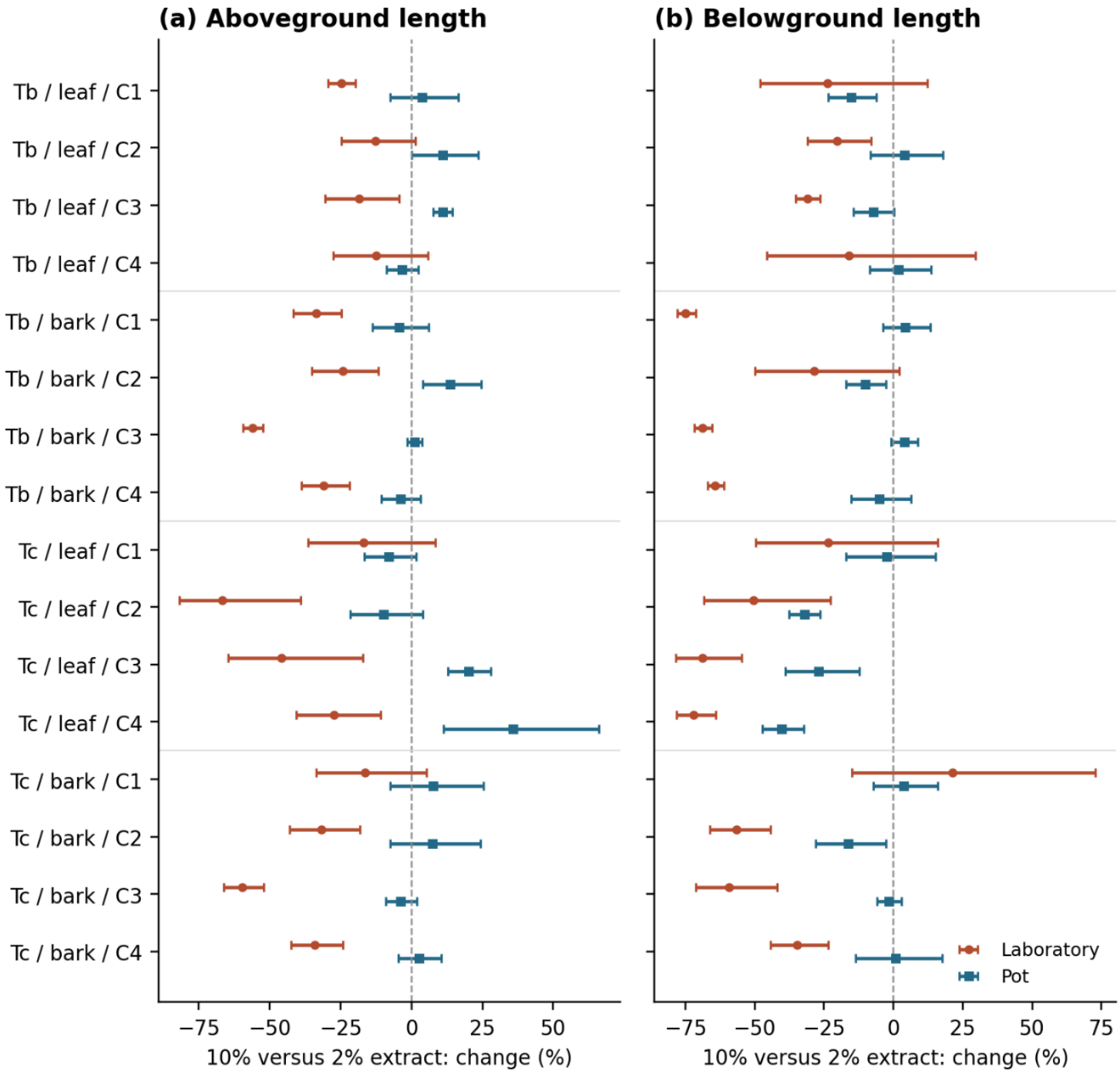



**Figure 2**

Crop- and organ-specific length responses to concentration escalation. Each estimate compares 10 with 2% and averages over the three extraction durations. Bars are pointwise approximate 95% Welch intervals. Tb, *T. bellirica*; Tc, *T. chebula*. C1–C4 retain the original crop codes; their name mapping is provisional. Laboratory circles and pot squares distinguish assays. Sign reversals are descriptive and should not be interpreted as significant without the exploratory multiplicity-adjusted tests in Online Resource 2. R records are analysed as source summaries, not individual seedlings

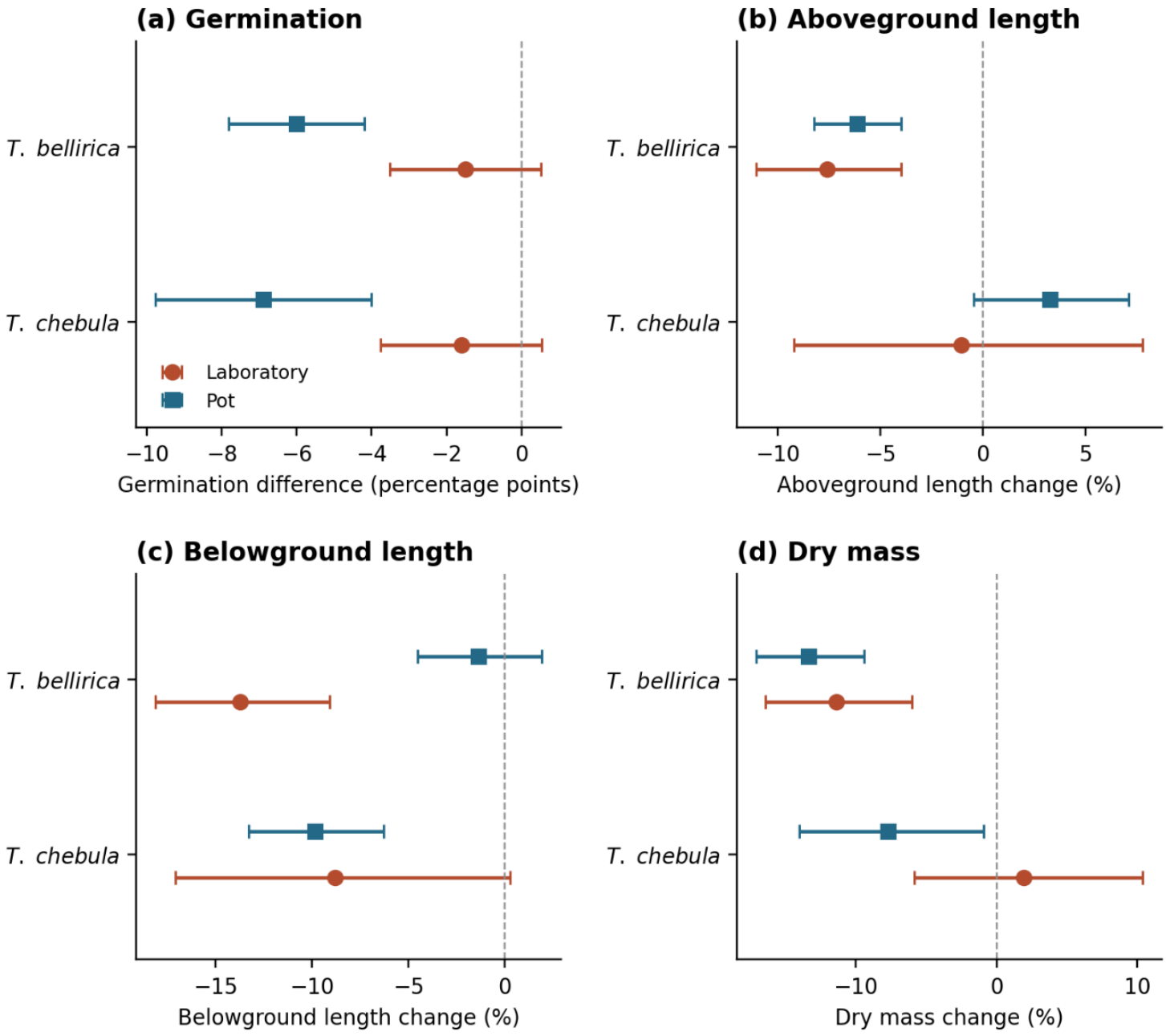



**Figure 3**

Responses to extending extract preparation from 24 to 72 h. Points average equally over the three concentrations, two donor organs and four crop codes within each donor and assay. Symbols, intervals and response scales follow Fig. 1. Preparation duration is distinct from seedling observation time. The plotted intervals are pointwise; multiplicity decisions use the 32 primary Holm-adjusted tests

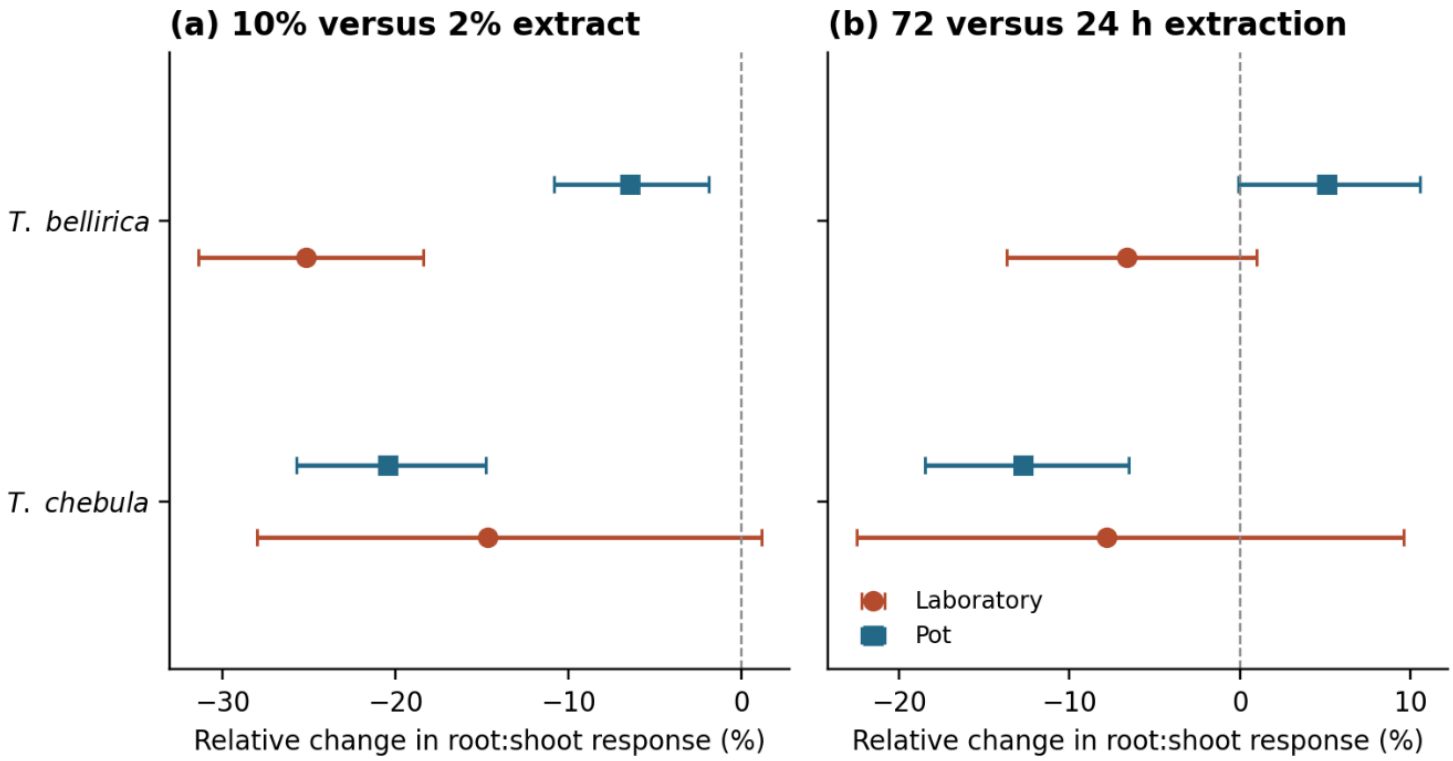



**Figure 4**

Relative root versus shoot response to (a) concentration escalation and (b) longer extraction. The difference between the marginal log-root and log-shoot contrasts is back-transformed as 100[exp(difference) − 1]. Negative values indicate that the root response declined more, or increased less, than the shoot response. Bars are approximate covariance-bounded sensitivity envelopes and are not guaranteed conservative 95% confidence intervals. This comparison does not establish individual-seedling allocation or a physiological trade-off

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DATAFILE.xlsx