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# Age-dependent physiological responses of *Corchorus olitorius* to aqueous extracts of *Murraya koenigii* and *Tithonia rotundifolia*

**Ogunwole, A. A.**

*Faculty of Science, Department of Biological Science, Lead City University, Ibadan, Nigeria*
*Author for correspondence: shardaylyd@gmail.com*

***Summary:*** Sustainable crop production increasingly utilizes allelopathic plants as sources of bioactive compounds, yet their bimodal inhibitory–stimulatory effects and oxidative mechanisms require further elucidation. This study investigated the concentration-, tissue-, and stage-dependent effects of aqueous shoot extracts from *Murraya koenigii* and *Tithonia rotundifolia* on the germination, growth, and oxidative defense of *Corchorus olitorius*. Seed emergence and elongation of radicles and plumules were monitored in the laboratory using 50% and 100% aqueous extracts, while potted seedlings were treated separately with 100% extracts. Laboratory bioassays demonstrated a dose-dependent response: a 50% *M. koenigii* extract transiently increased germination by 4%, while higher concentrations of both species inhibited germination by 7–25%. Juvenile growth inhibition was tissue-specific, with 100% *M. koenigii* primarily suppressing plumule elongation and 100% *T. rotundifolia* significantly reducing radicle growth. Conversely, pot experiments using 100% extract concentrations of both plants significantly enhanced vegetative growth, physiological traits, and biochemical constituents, including protein and ascorbic acid. These extracts bolstered the antioxidant defense system—increasing superoxide dismutase, catalase, and peroxidase activities—while markedly reducing malondialdehyde levels. These findings provide evidence of allelopathic hormesis, where initial inhibitory effects transition into growth stimulation and oxidative stress mitigation during later developmental stages. The results suggest that *M. koenigii* and *T. rotundifolia* shoot extracts serve as effective eco-friendly biostimulants that improve crop performance by modulating antioxidant responses.

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***Key words:*** allelopathy, oxidative stress, antioxidant enzymes, hormesis, natural bio-stimulant, allelochemicals, vegetable production

## Introduction

Indigenous vegetables are an indispensable part of the diet for both rural and urban dwellers in Africa. These crops combat malnutrition by providing a rich source of essential micronutrients, while offering smallholder farmers a fast-growing, reliable income source. *Corchorus olitorius* (L., Malvaceae), a tropical annual herb indigenous to Africa, is widely cultivated for culinary, fiber, and medicinal purposes. Its highly nutritious leaves contain significant amounts of protein, lipids, fiber, and carbohydrates, along with essential vitamins (A, B, C) and minerals (beta-carotene, calcium, magnesium, iron, and potassium) crucial for human growth (Aglinglo et al., 2022).

The intricate biochemical mediation of plant interactions, termed allelopathy, plays a pivotal role in shaping plant communities through the release of secondary metabolites (allelochemicals) that elicit divergent responses in neighboring flora (Rice, 1984). This mechanism holds significant promise for the development of sustainable agricultural practices that minimize reliance on synthetic inputs (Cheng & Cheng, 2015). Of particular interest is allelopathic hormesis, where sub-toxic concentrations of these metabolites function as signaling elicitors to enhance physiological vigor and prime plant antioxidant defense systems against oxidative stress (Belz et al., 2011).

The practical integration of allelopathy is multifaceted, involving both direct bio-interference for weed management and metabolic fortification of crops. Species like *Murraya koenigii* and *Tithonia rotundifolia* contain dense profiles of bioactive compounds (e.g., carbazole alkaloids, phenolics, sesquiterpene lactones) with known allelopathic activity (Schuster et al. 1992; Ningappa et al., 2008; Kato-Noguchi, 2020). Existing research often presents conflicting results: laboratory germination bioassays typically report inhibition, while field- or pot-based applications often observe growth-promoting effects, suggesting a concentration-dependent dual action (Oke & Aiyelaagbe, 2011; Ogunwole, 2026).

At the cellular level, allelopathic stress is closely linked to the modulation of reactive oxygen species (ROS) homeostasis and subsequent oxidative damage (Apel & Hirt, 2004). Plants counteract ROS toxicity via enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Gill & Tuteja, 2010). Enhanced activity of these enzymes serves as a critical biochemical indicator of improved stress tolerance and a potential mechanism for allelochemical-induced growth stimulation (Belz et al., 2011). Despite these insights, few studies have integrated laboratory-scale germination assays with pot-based physiological evaluations to clarify the transition from phytotoxicity to stimulation.

This study aims to address this gap by comprehensively investigating the concentration-, tissue-, and stage-dependent effects of aqueous shoot extracts from *M. koenigii* and *T. rotundifolia* on the germination, growth parameters, and enzymatic antioxidant defense responses of *Corchorus olitorius* L.

## Materials and methods

### *Study area and experimental site*

During the dry season (January to March 2021), pot and laboratory experiments were carried out at Wesley University, Ondo (7.5165° N, 4.8211° E). The pot trials took place outdoors near the Microbiology and Biochemistry Laboratory, with analytical work following in the Biochemistry Laboratory.

### *Sample collection*

Seeds of *Corchorus olitorius* were sourced from the Agricultural Development Programme (ADP) office at the Ondo West Local Government Secretariat. Plant materials were harvested from two distinct fallow sites located along Road 4 in the Ilupeju community, Jolaco area, Ondo. Collected samples included 2–4-week-old *Murraya koenigii* seedlings and the upper terminal portions (<10 cm) of 7–9-week-old *Tithonia rotundifolia*. All botanical samples were identified and authenticated before use.

### *Preparation of plant extracts*

The collected fresh, healthy shoots of *M. koenigii* and *T. rotundifolia* were washed thoroughly, separately chopped into small pieces, and homogenized in distilled water (1:1 w/v). The homogenate was incubated for 24 h at room temperature and then filtered sequentially through muslin cloth and Whatman No. 1 filter paper to obtain the stock solution (100% aqueous extract). A 50% concentration was prepared by diluting the stock with distilled water. Extracts were prepared freshly prior to use, following a method adapted from Kato-Noguchi (2020).

### *Laboratory germination bioassay*

Laboratory germination assays were conducted using standard Petri dish methods. Seeds of *Corchorus olitorius* were surface-sterilized using 1% sodium hypochlorite and rinsed thoroughly under running water. Twenty seeds were placed in Petri dishes lined with filter paper and moistened with 5 mL of the respective extract concentrations or distilled water (control). Treatments were replicated five times. Germination percentage was recorded, while plumule and radicle lengths were measured on the 7th day, according to the ISTA (2015) guidelines.

### *Pot experiment*

Only 100% aqueous extracts of *M. koenigii* and *T. rotundifolia* (hereafter referred to as MkE and TrE, respectively) were used in the pot experiment with homogenous topsoil (humus). Fifteen *C. olitorius* seeds were sown in each of sixty plastic pots (30 cm height × 20 cm diameter). Seedlings were watered twice daily for two weeks and subsequently thinned to 10 per pot to avoid overcrowding.

The two treatment groups consisted of separate applications of 300 mL each of the fresh shoot aqueous extracts: i) *M. koenigii* (MkE), and ii) *T. rotundifolia* (TrE). The control group received 300 mL of distilled water. The extracts or water (control) were applied twice daily as soil drenches.

Seedlings were grown under natural conditions in an experimental area fenced with wire mesh and netting to prevent pests such as rodents and insects. The experimental design was a Completely Randomized Design (CRD). Sampling was conducted immediately before the commencement of treatment application, and subsequent samplings occurred weekly for a period of six weeks.

### *Biochemical and antioxidant analyses*

Soluble protein content was determined using the micro-Kjeldahl nitrogen method (A.O.A.C., 2000). Ascorbic acid content was estimated following the dichlorophenolindophenol titrimetric method (A.O.A.C., 2005). Leaf proline content was determined following the procedures of Bates et al. (1973). Total soluble sugars (TSS) content was obtained using the Anthrone method (Irigoyen et al., 1992). Total phenol content was determined using the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007), and absorbance was measured at 760 nm using gallic acid as a reference standard. Total flavonoids in the leaf samples were determined using the aluminum chloride method, and absorbance was recorded at 510 nm using quercetin as a standard reference. Lipid peroxidation was estimated as malondialdehyde (MDA) content using the thiobarbituric acid method (Hodges et al., 1999).

### *Determination of antioxidant enzyme activities*

Fresh, healthy leaf samples (0.5 g) were harvested every week for six weeks and immediately flash-frozen in liquid nitrogen for transport to the laboratory for enzyme activity analysis. The fresh tissue was homogenized thoroughly in 10 mL of ice-cold extraction buffer using a pre-chilled pestle and mortar on ice. The buffer composition was 9 mL of 0.2 M potassium phosphate buffer (pH 7.0) and 1 mL of 0.1 M EDTA. The resulting homogenate was centrifuged at 15,000 rpm for 10 minutes at 4 °C. The supernatant, collected as the enzyme extract, was kept on ice until used for analysis.

Superoxide dismutase (SOD) activity was assayed based on the inhibition of nitro blue tetrazolium reduction and expressed as IU $min^{-1}$ $mg^{-1}$ FW (Giannopolitis & Ries, 1977). Peroxidase (POD) activity was measured using guaiacol oxidation and expressed as mM $min^{-1}$ $g^{-1}$ FW ($\epsilon$= 26.6 $mM^{-1}$ $cm^{-1}$) (Aebi & Lester, 1984). Catalase (CAT) activity was determined by monitoring the decomposition of hydrogen peroxide and expressed as mM $min^{-1}$ $g^{-1}$ FW ($\epsilon$ = 40 $mM^{-1}$ $cm^{-1}$) (Chance & Maehly, 1955).

### *Statistical analysis*

Data were subjected to one-way Analysis of Variance (ANOVA), and means were separated using the Honest Significant Difference (HSD) test at $p \leq 0.05$. Results are presented as the mean ±standard error (SE).

## Results

### *Effects of shoot aqueous extracts on germination and early seedling growth of Corchorus olitorius*

Aqueous shoot extracts of *Murraya koenigii* and *Tithonia rotundifolia* exerted concentration-dependent effects on the germination and early seedling growth of *C. olitorius* (***Figure 1***). At a 50% concentration, the *M. koenigii* extract resulted in a slight but measurable stimulation of germination, increasing the germination percentage by approximately 4% relative to the control. In contrast, the 50% *T. rotundifolia* extract reduced germination by about 7%. Increasing the extract concentration to 100% caused marked inhibition of germination in both species. Germination was reduced by 16% with the 100% *M. koenigii* extract application. The 100% *T. rotundifolia* extract produced the strongest inhibitory effect, reducing germination by approximately 25% relative to the control.

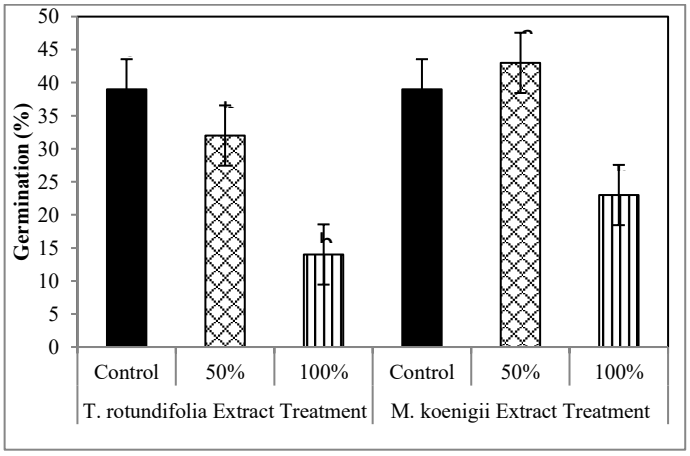


***Figure 1.*** Effects of shoot aqueous extracts of *Murraya koenigii* and *Tithonia rotundifolia* on germination percentage of *Corchorus olitorius* seeds. Bars represent mean values (n = 5). Bars with different letters are significantly different at $p \leq 0.05$.

Juvenile seedling growth responded differentially to the extracts (***Figure 2***). Plumule elongation was significantly reduced by the 100% *M. koenigii* extract, whereas radicle growth was more strongly inhibited by the 100% *T. rotundifolia* extract. Specifically, while the application of 100% MkE decreased *Corchorus* plumule growth by 65.9%, 100% TrE transiently decreased it by 19.5%. Conversely, 100% TrE reduced radicle extension by 57.4%, while 100% MkE reduced it by 13.2%. These responses indicate differential sensitivity of shoot and root tissues to the allelochemicals present in the two extracts.

### *Effects of shoot aqueous extracts on growth and physiological attributes of potted C. olitorius seedlings*

Application of 100% shoot aqueous extracts to potted *C. olitorius* seedlings resulted in a significant stimulation of vegetative growth compared with the control (***Table 1a-b***). Application of MkE induced significant increases in virtually all measured morphometrics of *Corchorus* seedlings, whereas TrE treatments promoted only a few of these attributes.

Specifically, the *M. koenigii* extract (MkE) significantly increased the following parameters: shoot height (SH) by 143.6%, leaf count (LN) by 120.9%, leaf area (LA) by 135.8%, root length (RL) by 122.4%, root:shoot ratio (RSR) by 36.4%, root fresh weight (RFW) by 159.0%, root biomass (RDW) by 109.1%, shoot fresh weight (SFW) by 87.3%, shoot biomass (SDW) by 58.9%, plant fresh weight (WPFW) by 95.9%, and plant biomass (WPDW) by 64.2%. In contrast, the *T. rotundifolia* extract (TrE) significantly augmented SH by 57.6%, LN by 23.9%, SFW by 55.7%, WPFW by 55.6%, RDW by 63.6%, and RSR by 21.8% (***Table 1a-b***).

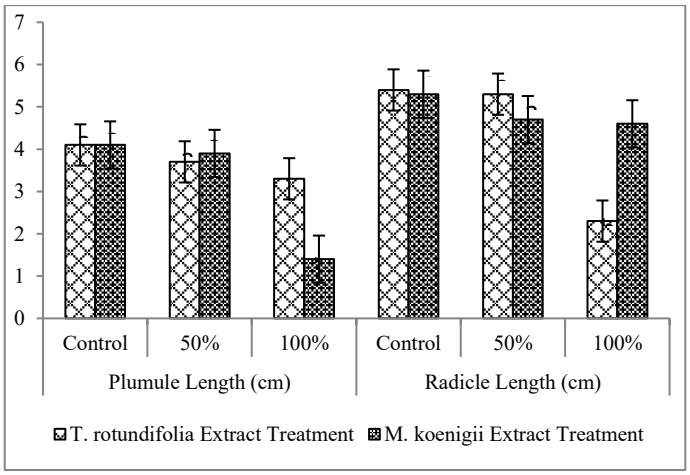


***Figure 2.*** Effect of shoot aqueous extracts of *Murraya koenigii* and *Tithonia rotundifolia* on plumule and radicle elongation of *Corchorus olitorius* seedlings. Bars represent mean values (n = 5). Bars with different letters are significantly different at $p \leq 0.05$.

Physiological performance of treated plants was also improved. Extract-treated seedlings exhibited higher overall vigor and improved physiological attributes relative to untreated controls, indicating positive growth modulation under pot conditions (***Table 2a-b***).

Relative to the control, separate treatments with extract of *M. koenigii* and *T. rotundifolia* slightly decreased the *Corchorus* leaf relative water content by 0.8% and 1.8%, respectively. However, the leaf area ratio of the seedlings increased by 48.9% with MkE and 2.4% with TrE (***Table 2a-b***).

The separate application of extracts of *M. koenigii* and *T. rotundifolia* caused a significant improvement in the photosynthetic pigments of the test crop. In *M. koenigii* extract-treated seedlings, the levels of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b), and carotenoids (Car) were significantly augmented by 324.4%, 202.3%, 265.0%, and 85.7%, respectively. Higher increases of 478.4%, 320.8%, 401.7%, and 267.3% were detected accordingly in the *T. rotundifolia* extract-treated seedlings. The Chl a/b ratio and Chl/Car ratio were 64.6% and 271.4% greater in *M. koenigii* extract-treated seedlings, respectively, and 32.4% and 29.9% greater in *T. rotundifolia*-treated seedlings (***Table 2a-b***).

### *Effects on biochemical constituents, antioxidant enzymes, and lipid peroxidation*

Biochemical analysis revealed that the application of 100% shoot aqueous extracts significantly increased soluble protein and ascorbic acid contents in *C. olitorius* leaves (***Table 3***). The crude protein percentage and ascorbic acid content were significantly higher in leaves treated with *M. koenigi* extract (51% and 89.5%, respectively) and TrE (37.6% and 25.1%, respectively) comparing to the control (***Table 3***).

***Table 1a.*** Growth attributes of potted *Corchorus olitorius* treated with shoot aqueous extracts of *M. koenigii* and *T. rotundifolia*

| Treatment | Shoot height (cm) | Leaf number | Leaf area (cm²) | Total leaf area (cm²) |
|---|---|---|---|---|
| Control | 11.94 ± 0.34$^{c}$ | 9.8 ± 0.37$^{c}$ | 9.98 ± 0.24$^{c}$ | 999.81 ± 4.50$^{c}$ |
| *M. koenigii* | 28.02 ± 0.19$^{a}$ | 21.4 ± 0.51$^{a}$ | 22.29 ± 0.27$^{a}$ | 5809.21 ± 10.43$^{a}$ |
| *T. rotundifolia* | 18.04 ± 0.17$^{b}$ | 12.2 ± 0.37$^{b}$ | 14.24 ± 0.17$^{b}$ | 1950.99 ± 0.50$^{b}$ |

Values are mean ± SE (n = 5). Different letters denote significance ($p \leq 0.05$).

***Table 1b.*** Growth attributes of potted *Corchorus olitorius* treated with shoot aqueous extracts of *M. koenigii* and *T. rotundifolia*

| Treatments | Root fresh weight (g) | Shoot fresh weight (g) | Plant fresh weight (g) | Root biomass (g) | Shoot biomass (g) | Plant biomass (g) |
|---|---|---|---|---|---|---|
| Control | 1.25 ± 0.04$^{c}$ | 8.03 ± 0.11$^{c}$ | 9.28 ± 0.13$^{c}$ | 0.11 ± 0.01$^{c}$ | 0.61 ± 0.02$^{c}$ | 0.72 ± 0.02$^{c}$ |
| *T. rotundifolia* | 1.62 ± 0.05$^{b}$ | 9.87 ± 0.14$^{b}$ | 11.49 ± 0.18$^{b}$ | 0.16 ± 0.01$^{b}$ | 0.82 ± 0.03$^{b}$ | 0.98 ± 0.03$^{b}$ |
| *M. koenigii* | 1.94 ± 0.06$^{a}$ | 11.24 ± 0.16$^{a}$ | 13.18 ± 0.21$^{a}$ | 0.21 ± 0.01$^{a}$ | 1.04 ± 0.04$^{a}$ | 1.25 ± 0.04$^{a}$ |

Values are mean ± SE (n = 5). Different letters indicate significant differences ($p \leq 0.05$).

***Table 2a.*** Physiological performance of potted *Corchorus olitorius* as influenced by extracts *M. koenigii* and *T. rotundifolia.*

| Treatment | Leaf area ratio | Relative water content (%) | Chlorophyll a (µM/g) | Total Chlorophyll (µM/g) |
|---|---|---|---|---|
| Control | 135.6 ± 3.2$^{c}$ | 71.8 ± 1.4$^{c}$ | 2.41 ± 0.06$^{c}$ | 3.53 ± 0.08$^{c}$ |
| *T. rotundifolia* | 152.3 ± 3.8$^{b}$ | 78.6 ± 1.6$^{b}$ | 3.18 ± 0.07$^{b}$ | 4.64 ± 0.09$^{b}$ |
| *M. koenigii* | 168.9 ± 4.1$^{a}$ | 85.9 ± 1.9$^{a}$ | 3.92 ± 0.09$^{a}$ | 5.76 ± 0.11$^{a}$ |

Values are mean ± SE (n = 5). Different letters denote significance ($p \leq 0.05$).

***Table 2b.*** Physiological performance of potted *Corchorus olitorius* as influenced by extracts *M. koenigii* and *T. rotundifolia*.

| Treatment | Chlorophyll a/b | Carotenoid (µM/g) | Chlorophyll/Carotenoid |
|---|---|---|---|
| Control | 2.15 ± 0.05$^{a}$ | 0.62 ± 0.02$^{c}$ | 5.69 ± 0.14$^{b}$ |
| *T. rotundifolia* | 2.18 ± 0.06$^{a}$ | 0.78 ± 0.03$^{b}$ | 5.95 ± 0.16$^{a}$ |
| *M. koenigii* | 2.13 ± 0.04$^{a}$ | 0.96 ± 0.03$^{a}$ | 6.00 ± 0.18$^{a}$ |

Values are mean ± SE (n = 5). Different letters denote significance ($p \leq 0.05$).

***Table 3.*** Effects of shoot aqueous extracts of *M. koenigii* and *T. rotundifolia* on biochemical constituents of potted *C. olitorius.*

| Treatments | Crude protein (%) | Ascorbic acid (mg 100 g-1) | Proline (mg g-1 FW) | Phenols (mg GAE g-1 DW | Flavonoids (mg QE g-1 DW) | Total soluble sugar (g 100 g-1 DW) |
|---|---|---|---|---|---|---|
| Control | 14.07 ± 0.07$^{c}$ | 3.92 ± 0.08$^{c}$ | 1.44 ± 0.01$^{c}$ | 298.4 ± 5.6$^{c}$ | 5.91 ± 0.12$^{c}$ | 35.2 ± 0.9$^{c}$ |
| *T. rotundifolia* | 27.64 ± 0.01$^{a}$ | 4.78 ± 0.09$^{b}$ | 3.16 ± 0.06$^{a}$ | 336.7 ± 6.4$^{b}$ | 6.84 ± 0.15$^{b}$ | 41.6 ± 1.1$^{b}$ |
| *M. koenigii* | 22.89 ± 0.01$^{b}$ | 5.86 ± 0.11$^{a}$ | 2.23 ± 0.01$^{b}$ | 372.9 ± 7.2$^{a}$ | 7.92 ± 0.18$^{a}$ | 48.3 ± 1.3$^{a}$ |

Values are mean ± SE (n = 5). Different letters denote significance ($p \leq 0.05$).

***Table 4.*** Antioxidant enzyme activities and lipid peroxidation in the potted *C. olitorius as influenced by extracts of M. koenigii and T. rotundifolia*.

| Treatment | Superoxide dismutase (SOD) (IU min$^{-1}$ mg$^{-1}$ FW) | Peroxidase (POD) (mM min$^{-1}$ g$^{-1}$ FW) | Catalase (CAT) (mM min$^{-1}$ g$^{-1}$ FW) | Malondialdehyde (MDA) content (nmol g$^{-1}$ FW) |
|---|---|---|---|---|
| Control | 0.115 ± 0.006$^{c}$ | 52.14 ± 0.07$^{c}$ | 16.28 ± 0.01$^{c}$ | 85.56 ± 0.04$^{a}$ |
| *M. koenigii* | 0.163 ± 0.006$^{a}$ | 93.73 ± 0.05$^{a}$ | 24.09 ± 0.07$^{a}$ | 42.11 ± 0.03$^{c}$ |
| *T. rotundifolia* | 0.154 ± 0.005$^{b}$ | 81.25 ± 0.06$^{b}$ | 22.17 ± 0.05$^{b}$ | 55.34 ± 0.02$^{b}$ |

Values are mean ± SE (n = 5). Different letters indicate significant differences ($p \leq 0.05$).

Levels of non-enzymatic antioxidants were also elevated in treated seedlings relative to the control (***Table 3***). Proline levels increased by 151.7% (MkE) and 132.8% (TrE), while total soluble sugars increased significantly by 97.2% with MkE treatment (***Table 3***). Flavonoid accumulation was induced by 47% (MkE) and 83.2% (TrE), and phenolic acids were enhanced by 67.9% (MkE) and 83.9% (TrE).

Activities of antioxidant enzymes were markedly enhanced by both extracts (***Table 4***). Superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (POD) activities were significantly higher in extract-treated potted plants than in the control. In MkE-treated seedlings, activities increased by 49.5%, 78.5%, and 44.2%, respectively, while in TrE-treated seedlings, increases of 44.1%, 53.4%, and 35.2% were observed, respectively (***Table 4***).

Concurrently, malondialdehyde (MDA) content was significantly reduced in both MkE- and TrE-treated seedlings relative to the control, indicating reduced lipid peroxidation and oxidative stress (***Table 4***).

## Discussion

Laboratory germination assays revealed clear concentration-dependent allelopathic responses of *C. olitorius* to shoot aqueous extracts of *M. koenigii* and *T. rotundifolia*. The 50% *M. koenigii* extract transiently promoted germination by 4%, suggesting a mild stimulatory or hormetic effect at sub-lethal concentrations (Belz et al., 2011). In contrast, germination was inhibited by 7% and 25% under 50% and 100% *T. rotundifolia* extracts, respectively, while the 100% *M. koenigii* extract reduced germination by 16%. Subtain et al. (2014) stated that allelochemicals restrain seed germination and development at higher concentrations and enhance the same at their lower concentrations. Thus, higher concentrations of allelochemicals are essential ecofriendly organic weedicides (Farooq et al., 2020), and at lower concentrations, allelochemicals may be used as growth promoters (Ain et al., 2023). These results confirm the stronger phytotoxic potential of *T. rotundifolia* and demonstrate that allelopathic responses are highly concentration-sensitive. A similar finding was reported by Otusanya et al. (2019).

Seedling growth responses further indicated tissue-dependent sensitivity. The significant inhibition of plumule elongation by the 100% *M. koenigii* extract suggests greater shoot sensitivity to its allelochemicals, whereas the marked suppression of radicle growth by the 100% *T. rotundifolia* extract indicates stronger root-targeted toxicity. Such differential effects likely reflect variations in the chemical composition and uptake pathways of the allelochemicals.

Despite laboratory inhibition at full strength, the pot experiment demonstrated that 100% shoot extracts were stimulatory to established *C. olitorius* seedlings. This apparent contradiction between laboratory phytotoxicity and pot-scale stimulation supports the concept of stage-dependent allelopathic action. While germinating seeds are highly vulnerable to allelochemical stress, established seedlings can utilize low-level oxidative signals induced by allelochemicals to activate defense and metabolic pathways (Farooq et al., 2013). Treated *Corchorus* plants exhibited improved growth and physiological attributes, accompanied by increased protein and ascorbic acid contents. Thus, aqueous extracts of *M. koenigii* and *T. rotundifolia* is apparently non-phytotoxic, and when applied to vegetable crops, can result in an economical, efficient, and professional method to improve crop productivity and to endorse the growth and development of vegetables.

The observed biphasic response aligns with the concept of allelopathic hormesis, whereby low doses of allelochemicals act as biochemical elicitors, priming antioxidant defenses, while high doses induce oxidative stress and growth inhibition (Belz et al., 2011; Cheema et al., 2012). This dual behavior explains why allelopathic plants can suppress weeds under laboratory screening yet promote crop performance when carefully managed in controlled or semi-field systems. Importantly, the consistency between laboratory inhibition and pot-scale stimulation highlights the need for dose optimization when deploying allelopathic extracts in crop production.

Enhancement of non-enzymatic antioxidants, together with significant increases in SOD, CAT, and POD activities, indicates the activation of antioxidant defense pathways. SOD catalyzes the dismutation of superoxide ($O^{\cdot -2}$) to hydrogen peroxide ($H_2O_2$), which is subsequently detoxified by CAT and POD. Enhanced activity of these enzymes serves as a critical biochemical indicator of improved stress tolerance (Gill & Tuteja, 2010). Therefore, elevated SOD activity suggests improved scavenging of superoxide radicals, while increased CAT and POD activities indicate efficient detoxification of hydrogen peroxide. These enzymatic responses collectively point to reduced oxidative damage and enhanced stress tolerance in treated seedlings. The findings further emphasize the relevance of antioxidant enzymes (SOD, POD, CAT) as sensitive biomarkers for assessing physiological responses to plant-derived bioactive compounds.

Concurrent reduction in MDA content confirms effective mitigation of oxidative stress and protection of membrane integrity. The results clearly demonstrate that shoot aqueous extracts of *M. koenigii* and *T. rotundifolia* act as biochemical elicitors, enhancing ROS scavenging capacity (Kato-Noguchi & Kato, 2025) and improving the physiological performance of *C. olitorius* at the seedling stage. Cheema et al. (2012) suggested that the crop growth-enhancing ability of sorghum water extract under sub-optimal conditions might be attributed to delayed leaf senescence, and the scavenging of reactive oxygen species (ROS). Foliar application of sorghum water extract enhances membrane stability, water relation, and grain yield of maize at low concentrations (3%, 5% and 10%) (Kamran et al., 2016, 2019).

## Conclusions

Shoot aqueous extracts of *M. koenigii* and *T. rotundifolia* exhibit pronounced stage-dependent allelopathic effects on *C. olitorius*. Under laboratory conditions, higher concentrations inhibited germination and early seedling growth. Conversely, application to established seedlings stimulated growth, enhanced physiological performance and biochemical constituents, bolstered antioxidant defense systems, and mitigated oxidative damage. These results demonstrate the dual, hormetic potential of these allelopathic plants. They can function as both natural weed suppressants and physiological stimulants, depending critically on the application strategy. The findings support their judicious use as natural biostimulants and eco-friendly weed management tools in sustainable agriculture. Careful optimization of the extract concentration and application strategy is essential to maximize agronomic benefits while avoiding potential phytotoxicity. The integration of laboratory screening with pot experiments provides a robust framework for identifying safe and effective extract concentrations for future development as eco-friendly inputs for sustainable crop production

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