

**ALLELOPATHIC EFFECTS OF *Tithonia diversifolia* N-BUTANOLIC SUB-FRACTIONS ON WEED SUPPRESSION, GERMINATION, AND CYTOTOXICITY IN TWO COWPEA VARIETIES**

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ABSTRACT

The search for sustainable alternatives to synthetic herbicides has intensified interest in plant-derived allelochemicals for weed management. This study evaluated the effects of *Tithonia diversifolia* n-butanolic sub-fractions on weed suppression indices, germination, seedling growth, and cytotoxicity in two varieties of *Vigna unguiculata* (Vital-5 and K-28). Germination was significantly reduced in all treated groups compared to the control, with sub-fraction G exhibiting the strongest inhibitory effect, particularly in Vital-5, while K-28 showed relatively higher tolerance. Seedling growth responses revealed consistent inhibition of radicle elongation across treatments, whereas some sub-fractions (E and H) stimulated plumule growth in K-28, suggesting a hormetic response. All sub-fractions demonstrated measurable weed suppression, with G showing the highest suppression and E exhibiting the greatest weed suppression efficiency. Cytological analyses revealed pronounced mitotic abnormalities, including micronuclei formation, chromosomal fragmentation, spindle disruption, and complete mitotic arrest, indicating strong cytotoxic effects. Overall, the findings highlight the significant allelopathic and bioherbicidal potential of *T. diversifolia* n-butanolic sub-fractions. However, the observed phytotoxicity to cowpea underscores the need for optimization to achieve selective weed control in sustainable agricultural systems.

Keywords: Allelopathy, Weed Suppression, *Vigna Unguiculata*, Germination Inhibition, Cytotoxicity, Seedling Growth, Bioherbicide

INTRODUCTION

Sustainable weed management remains a major challenge in agricultural systems, particularly in sub-saharan Africa where reliance on synthetic herbicides is often constrained by cost, environmental concerns and the emergence of herbicide-resistant weed species. Consequently, attention has increasingly shifted toward plant-derived bioactive compounds with allelopathic potential as eco-friendly alternatives for weed suppression. Allelopathy refers to the production and release of secondary metabolites (allelochemicals) by plants that influence the germination, growth, and development of neighbouring plants (Latif et al., 2017). These allelochemicals may inhibit seed germination, disrupt cellular processes, and ultimately suppress plant establishment, making them valuable tools in integrated weed management strategies (Cheng & Cheng, 2015; Scavo et al., 2018). Among such plants, *Tithonia diversifolia* (Hemsl.) A. Gray belonging to the Asteraceae family and commonly known as Mexican sunflower, has attracted considerable scientific interest due to its rapid growth, high biomass production, and rich phytochemical composition. Native to Central America but now widely distributed across tropical regions, *T. diversifolia* has been reported to contain diverse secondary metabolites including flavonoids, sesquiterpene lactones, phenolics, and diterpenoids, many of which exhibit allelopathic, antimicrobial, and cytotoxic properties (Olabode et al., 2007; Ajao et al., 2017). The plant is also widely utilized in Africa as green manure due to its ability to improve soil

fertility and nutrient availability, but its bio-herbicidal potential remains underexplored relative to its agronomic applications.

Recent studies have demonstrated that extracts and fractions of *T. diversifolia* can significantly influence seed germination and seedling growth in target plant species, suggesting strong allelopathic activity. The magnitude of these effects is often dependent on extract concentration, solvent polarity, and the sensitivity of the recipient species (Inderjit & Duke, 2003; Scavo et al., 2018). Organic solvent fractions, particularly n-butanolic fractions, are known to concentrate moderately polar bioactive compounds, which may enhance phytotoxic effects compared to crude extracts. Such fractions therefore provide a promising avenue for isolating potent allelochemicals with selective weed suppression capabilities. Cowpea (*Vigna unguiculata* (L.) Walp.) is an economically important legume crop widely cultivated in tropical and subtropical regions, including Nigeria, where it serves as a major source of dietary protein and income for smallholder farmers. However, cowpea production is severely affected by weed competition, especially during early growth stages, leading to significant yield losses (Yadav et al., 2017). While exploring plant-based herbicides, it is essential to evaluate not only weed suppression efficacy but also potential phytotoxic or cytotoxic effects on the crop itself to ensure selectivity and safety.

Weed suppression indices, including germination inhibition percentage, seedling vigor index, and relative growth

reduction, provide quantitative measures of allelopathic impact and are widely used to assess the bio-herbicidal potential of plant extracts (Dayan et al., 2009). In addition, cytotoxicity assays such as root growth inhibition and mitotic index evaluation offer insight into the cellular mechanisms underlying phytotoxic effects, including disruption of cell division and membrane integrity (Nicuță et al., 2025). Integrating these approaches enables a comprehensive assessment of both ecological and physiological impacts of allelopathic agents.

Despite growing evidence on the allelopathic properties of plant extracts, there remains limited information on the specific effects of *T. diversifolia* n-butanolic fractions on weed suppression indices and their concurrent impact on germination and cytotoxic responses in *V. unguiculata*. Understanding these interactions is critical for developing plant-based herbicides that are both effective against weeds and safe for crop plants.

Therefore, this study aims to evaluate the effect of *T. diversifolia* n-butanolic sub-fractions on weed suppression indices, as well as its influence on seed germination and cytotoxicity in *Vigna unguiculata*. The findings are expected to contribute to the development of sustainable, plant-derived weed management strategies suitable for resource-limited agricultural systems.

MATERIALS AND METHODS

Study Area and Experimental Design

The experiment was conducted at the Al-Hikmah University Botanical Garden, Ilorin, Kwara State, Nigeria, located within the Southern Guinea Savanna ecological zone characterized by distinct wet and dry seasons. Prior to the 2025 planting season, fertile soil containing natural weed flora was randomly collected, homogenized, and apportioned into experimental containers at 225.50 g per container. The soil samples were subsequently treated with N-butanolic sub-fractions (C–H) of *Tithonia diversifolia* to evaluate their effects on weed suppression, seed germination, and cytological toxicity.

Plant Materials and Extraction Procedure

Seeds of cowpea (*Vigna unguiculata* L. Walp.), varieties Vital-5 and K-28, were sourced locally. Fresh leaves of *Tithonia diversifolia* were collected along Air Force Road, Oloje, Ilorin (Latitude: 8.510426; Longitude: 4.512863), and authenticated for taxonomic accuracy. The leaves were air-dried for seven days to minimize enzymatic degradation and preserve phytochemical constituents, pulverized into fine powder, and sieved (Kabir, 2024; Peiris et al., 2023). Aqueous extraction was carried out by soaking the powdered material in distilled water for 24 h with intermittent stirring, followed by filtration using Whatman No.1 filter paper. The filtrate was subsequently lyophilized at -60 °C to preserve phytochemical integrity and stability (Lalchandani et al., 2025). Solvent partitioning was then performed using n-butanol (600 mL) and aqueous extract (400 mL) to isolate semi-polar bioactive constituents (Zaky et al., 2024; Sun et al., 2025). The extract was further fractionated using silica gel column chromatography with gradient elution to obtain sub-fractions C–H. Fractionation was monitored by thin-layer chromatography (TLC), consistent with standard phytochemical separation techniques (Ghenabzia et al., 2023).

The sub-fractions were retrieved and stored for the bioassays. The absorbance of all retrieved sub-fractions were obtained at 600nm wavelength were 0.574, 0.685, 0.422, 0.240, 2.125, and 1.978 for sub-fractions C, D, E, F, G and H respectively.

Treatment Application and Planting

Seven treatment groups (sub-fractions C–H and a control) were established. The sub-fractions were diluted in 250 mL distilled water and applied using a hand sprayer to ensure uniform distribution. Viable cowpea seeds were manually sown at a depth of 3 cm, with one seed per hole. Each treatment, including the control, was replicated five times.

Germination and Growth Parameters

Germination percentage, plumule length, and radicle length were measured at specified intervals. Germination percentage was calculated as the proportion of germinated seeds relative to the total number of seeds sown.

Weed suppression efficiency was determined by comparing weed biomass in treated and control pots using the equation:

$$\text{Weed Suppression (\%)} = \frac{\text{Control Value} - \text{Treatment Value}}{\text{Control Value}} \times 100$$

Cytological Analysis

Root tips were harvested for cytological examination and pre-treated in colchicine for 1.5 h to arrest cells at metaphase. The samples were rinsed and fixed in a solution of glacial acetic acid and ethanol (1:3, v/v) for 20 min. Root tips were hydrolyzed in 1 N HCl at 60 °C for 5 min to soften the tissues, a standard approach for enhancing chromosomal visibility. The tissues were stained with 2% aceto-carmin and squashed for microscopic observation.

RESULTS AND DISCUSSION

The effects of *Tithonia diversifolia* n-butanolic sub-fractions on germination, seedling growth, weed suppression indices, and cytotoxicity in *Vigna unguiculata* revealed pronounced allelopathic activity with clear variation among fractions and between cowpea varieties.

Germination Response

Germination of *Vigna unguiculata* was significantly reduced by all sub-fractions compared to the control, indicating strong inhibitory effects of the bioactive constituents. In the Vital-5 variety, the control consistently recorded the highest germination percentage across all observation periods, while sub-fraction G exhibited the most pronounced inhibition, followed by F and H. This trend suggests that these fractions contain potent allelochemicals capable of disrupting early germination processes such as water uptake, enzyme activation, and mobilization of seed reserves (Scavo et al., 2018; Cheng & Cheng, 2015). In contrast, the K-28 variety demonstrated relatively higher tolerance to the treatments. Although the control still recorded the highest germination, sub-fraction H consistently ranked second, while sub-fraction E exhibited the strongest inhibitory effect. The observed differences between the two varieties indicate a genotype-dependent response to allelochemical stress, likely influenced by differences in seed physiology, permeability, and detoxification capacity (Inderjit & Duke, 2003; Scavo et al., 2018).

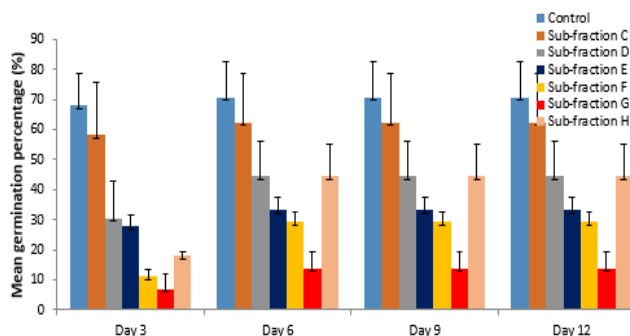


Figure 1: Effects of *Tithonia diversifolia* N-Butanolic Fraction on Germination Rate of Cowpea (Vital-5)

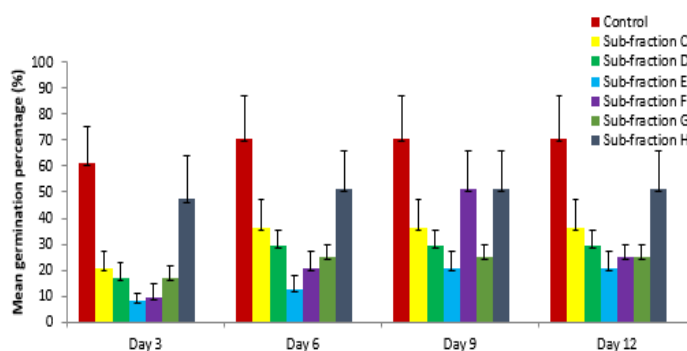


Figure 2: Effects of *Tithonia diversifolia* N-Butanolic Fraction on Germination Rate of Cowpea (Var. K-28)

Seedling Growth Performance

The inhibitory effects of the sub-fractions extended to seedling growth parameters. In Vital-5, both plumule and radicle lengths were significantly reduced in all treatments relative to the control, with sub-fraction F showing the strongest suppression. This suggests that the allelochemicals interfere with cell division and elongation processes essential for early seedling development (Dayan et al., 2009). In K-28, however, a differential response was observed. While radicle growth was consistently reduced across all treatments, some

sub-fractions (notably E and H) enhanced plumule elongation beyond control levels. This phenomenon suggests a hormetic response, where low to moderate levels of stress-induced compounds stimulate shoot growth while still inhibiting root development (Siemieniuk et al., 2025; Scavo et al., 2018). The greater sensitivity of radicles compared to plumules may be attributed to their direct exposure to the allelochemicals and their higher metabolic activity during early growth (Cheng & Cheng, 2015).

Table 1: Effects of *Tithonia Diversifolia* N-Butanolic Fraction on Plumule and Radicle Length of Cowpea (Vital-5)

Sub-fraction	Plumule (cm)	Radicle (cm)
Control	2.9667 ± 0.61 ^a	3.6000 ± 0.61 ^a
Sub-fraction C	1.7333 ± 0.32 ^c	2.5333 ± 0.33 ^c
Sub-fraction D	1.3000 ± 0.25 ^d	2.3667 ± 0.34 ^d
Sub-fraction E	1.1000 ± 0.38 ^e	2.1667 ± 0.17 ^e
Sub-fraction F	0.6667 ± 0.44 ^g	1.7000 ± 0.91 ^g
Sub-fraction G	0.9333 ± 0.27 ^f	2.0333 ± 0.39 ^f
Sub-fraction H	2.1000 ± 0.46 ^b	2.9667 ± 0.27 ^b
Total	1.6857 ± 0.39	2.4810 ± 0.43

Values are mean ± SEM, n = 5; Sub-fraction C = 0.574, Sub-fraction D = 0.685; Sub-fraction E = 0.422; Sub-fraction F = 0.240; Sub-fraction G = 2.125; and Sub-fraction H = 1.978.

Absorbance of sub fractions use were taken at 600nm wavelength. Mean with the same superscript are statistically the same at p ≤ 0.05.

Table 2: Effects of *Tithonia Diversifolia* N-Butanolic Fraction on Plumule and Radicle Length of Cowpea (Var. K-28)

Sub-fraction	Plumule	Radicle
Control	1.30 ± 0.25 ^g	3.60 ± 0.59 ^a
Sub-fraction C	1.37 ± 0.18 ^f	3.03 ± 0.24 ^b
Sub-fraction D	1.40 ± 0.31 ^e	2.50 ± 0.26 ^c
Sub-fraction E	2.40 ± 0.85 ^a	2.87 ± 0.44 ^c
Sub-fraction F	1.97 ± 0.26 ^c	2.17 ± 0.09 ^f

Sub-fraction	Plumule	Radicle
Sub-fraction G	1.50 ± 0.15 ^d	2.10 ± 0.26 ^g
Sub-fraction H	2.03 ± 0.23 ^b	2.77 ± 0.22 ^d
Total	11.97 ± 2.23	19.03 ± 2.10

Values are mean ± SEM, n = 5; Sub-fraction C = 0.574, Sub-fraction D = 0.685; Sub-fraction E = 0.422; Sub-fraction F = 0.240; Sub-fraction G = 2.125; and Sub-fraction H = 1.978. Absorbance of sub fractions use were taken at 600nm wavelength. Mean with the same superscript are statistically the same at $p \leq 0.05$.

Weed Suppression and Efficiency

All treated samples exhibited measurable weed suppression, whereas no suppression was observed in the control, confirming the herbicidal potential of the fractions. Sub-

fraction G recorded the highest weed suppression, closely followed by C, F, and D, indicating strong phytotoxicity against competing plant species. Interestingly, weed suppression efficiency was highest in sub-fraction E, followed closely by C and F. This suggests that while some fractions are more effective at inhibiting germination (e.g., G), others may exert stronger effects on overall weed growth and vigour. These findings indicate that the bioactivity of *T. diversifolia* fractions is multifaceted and likely due to a combination of different allelochemicals with varying modes of action (Dayan et al., 2009; Scavo et al., 2018).

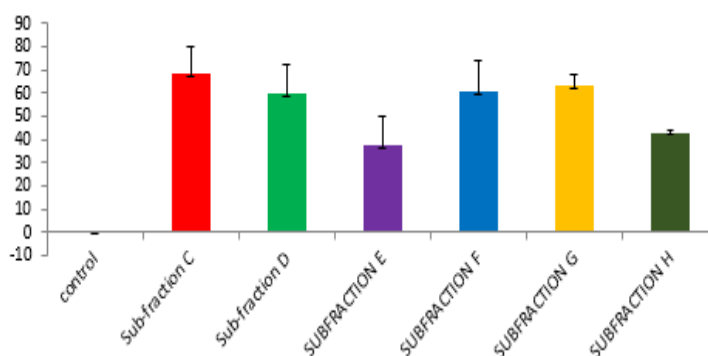


Figure 3: Effects of *Tithonia diversifolia* n-butanolic Fraction on Weed Control Efficiency of Cowpea (Vital-5)

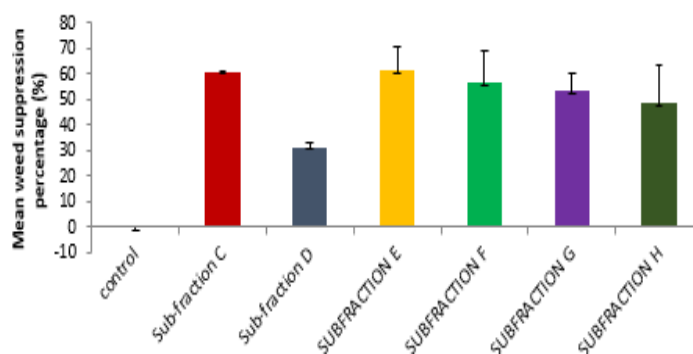


Figure 4: Effects of *Tithonia Diversifolia* N-Butanolic Fraction on Weed Control Efficiency of Cowpea (Var. K-28)

Cytotoxic Effects

Cytological analyses revealed significant disruptions in mitotic activity and chromosome integrity in treated root meristems, providing mechanistic insight into the observed growth inhibition. Various chromosomal abnormalities were recorded, including micronuclei formation, chromosomal stickiness, laggards, vagrant chromosomes, multipolar spindles, C-mitosis, and chromosome fragmentation. Sub-fraction D exhibited severe cytotoxicity, completely inhibiting cell division in one variety and inducing chromosomal stickiness and laggards in another, suggesting a possibility of genotoxicity. Sub-fractions E and F caused complete mitotic arrest, likely due to interference with DNA replication or activation of cell cycle checkpoints. Sub-fraction G induced C-mitosis and micronuclei formation,

indicating disruption of spindle apparatus and chromosome segregation. Sub-fraction H produced extensive chromosomal fragmentation and clumping, representing one of the most severe forms of genetic damage. The presence of micronuclei and fragmented chromosomes indicates DNA damage and genomic instability, while spindle abnormalities point to disruption of microtubule organization. Collectively, these effects demonstrate that the inhibitory action of the fractions is mediated not only through physiological interference but also through direct cytotoxic mechanisms (Nicuță et al., 2025; Leme & Marin-Morales, 2009). The control samples exhibited normal mitotic stages with intact chromosomes, confirming that the observed abnormalities were induced by the treatments and validating the reliability of the experimental system.

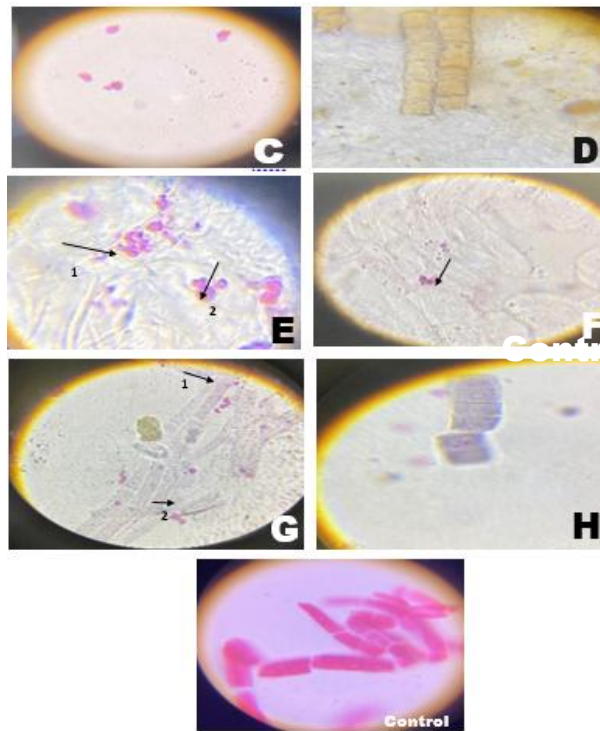


Plate 1: (C-H): Micrographs of Chromosomal Abnormalities Observed at Different Mitotic Stages of Meristematic Cells of *Vigna unguiculata* (Vital 5) Root Tips Induced by 8ppm of all Sub-fractions

Magnification = x100

C: Micronuclei; **D:** No cell Division; **E:** C-Mitosis¹, Multipolar spindles²; **F:** Vagrant chromosome; **G:** Disturbed

Metaphase¹; Fragmented Chromosome²; **H:** Fewer Cell Division; **Control:** normal division

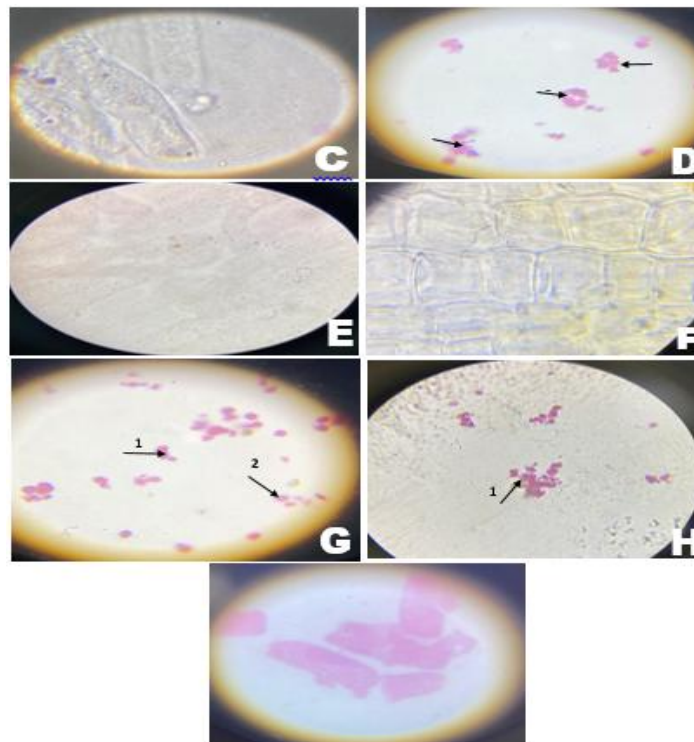


Plate 7: Micrographs of Chromosomal Abnormalities Observed at Different Mitotic Stages of Meristematic Cells of *Vigna unguiculata* (K-28) Root Tips Induced by 8ppm of all Sub-fractions

Magnification = x100

C: Fewer cell division; **D:** Stickiness and Disturbance at Metaphase¹; Laggards²; Disturbed Metaphase³; **E:** No cell division; **F:** No cell division; **G:** C-Mitosis¹; Micronuclei²; **H:** Fragmented and Clumping Chromosome¹; **Control:** normal division

Implications for Bio-herbicidal Application

The combined results demonstrate that *Tithonia diversifolia* n-butanolic sub-fractions possess strong allelopathic and bio-herbicidal properties, as evidenced by reduced germination, suppressed seedling growth, and effective weed control. However, the concurrent cytotoxic effects on *Vigna unguiculata* highlight a lack of selectivity, which may limit direct application without modification.

The variation in activity among sub-fractions suggests the presence of distinct groups of bioactive compounds with different mechanisms of action. While sub-fractions such as G and F are highly inhibitory, others like E and H exhibit mixed effects, including growth stimulation and cytotoxicity. This indicates the potential for selective isolation and formulation of active compounds to optimize weed control while minimizing crop toxicity (Dayan et al., 2009; Cheng & Cheng, 2015).

CONCLUSION

Overall, the study demonstrates that *Tithonia diversifolia* n-butanolic sub-fractions exert significant, fraction-dependent allelopathic effects on *Vigna unguiculata*. These effects include inhibition of germination, suppression of seedling growth, induction of cytotoxic abnormalities and effective weed suppression. While these properties underscore the potential of *T. diversifolia* as a source of natural herbicides, further refinement is required to enhance selectivity and ensure safe application in crop production systems.

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