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Assessment of the Phytotoxic Effects of the Herbicide Metsulfuron-Methyl on Cowpea at the Morphological and Biochemical Levels

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Abstract

Herbicides like sulfonylureas are widely used, but they can persist in the soil and harm unintended plants. In this study, we examined how metsulfuron-methyl (MSM) affects cowpea (*Vigna unguiculata*) by measuring changes in plant growth and chemical processes. We focused on seed germination and early seedling growth. We tested four MSM concentrations: 0, 100, 200, and 300 mg per liter. For the germination test, we used Petri dishes and treated healthy, sterilized seeds with these concentrations. For other growth and biochemical tests, we used plastic cups in which we sowed uniform healthy seeds after surface sterilization. We applied foliar sprays of MSM at different concentrations to 15-day-old plants at the three-leaf stage. We applied the leaf sprays when plants had three leaves, 15 days after planting. We measured plant growth and leaf green pigment 30 days after planting. We analyzed the data using one-way ANOVA and Tukey's HSD test at $P \leq 0.05$. MSM application significantly inhibited germination, vegetative growth, and chlorophyll accumulation in a concentration-dependent manner. Germination rates declined from 93% in the control group to 66% at 300 mg L⁻¹. Furthermore, shoot length decreased by 68% at 300 mg L⁻¹ relative to the control. Chlorophyll levels declined in MSM-treated plants. Treatment with 200 mg L⁻¹ resulted in a 53% reduction in chlorophyll a and up to a 60% reduction in chlorophyll b compared to untreated plants. Total chlorophyll content was also reduced by 55% at 200 mg L⁻¹ MSM relative to the control. Notably, plants treated with 300 mg L⁻¹, which were already desiccated at the time of sampling, exhibited no detectable chlorophyll. A similar case was also reported for biomass accumulation: no fresh biomass was observed at 30 mg L⁻¹ MSM because the plant was already dry. As herbicide concentration increased, root growth, leaf number, and leaf area all dropped significantly. This shows that cowpea is highly sensitive to MSM during the early seedling stage. The results indicate that herbicide application can pose risks to non-target plant organs. To promote sustainable plant growth, clear criteria and precautions should be established to safeguard these organs from adverse effects.

Keywords: Chlorophyll, Cowpea, Herbicide, Metsulfuron-methyl, Sulfonylurea

1. Introduction

Cowpea (*Vigna unguiculata*) is an important grain legume recognized for its significant economic and nutritional contributions to tropical and subtropical agro-ecosystems (Boukar *et al.*, 2019; Carvalho *et al.*, 2022). Its high protein and carbohydrate content, along with essential micronutrients, drives the

widespread cultivation of cowpea. Moreover, it shows strong resilience in extreme environments, making it a favorable crop for cultivation. Cowpea serves as an affordable source of plant-based protein and plays a significant role in addressing protein-energy malnutrition and food insecurity. It constitutes a staple food in sub-Saharan Africa, South Asia, and parts of Latin America (Abate *et al.*, 2011). Beyond its nutritional value, it has another crucial attribute: it forms a symbiotic relationship with nitrogen-fixing bacteria, which enhance soil fertility and support sustainable agricultural practices (Peoples *et al.*, 2021). Nevertheless, these traits and ongoing breeding initiatives have not increased yields to their genetic potential. According to the report, average cowpea yields in several regions barely exceed 400 kg ha⁻¹, whereas attainable yields should be between 2 and 2.5 tonnes per hectare, well below their potential (Boukar *et al.*, 2013). Weeds in agricultural fields are a major biotic constraint that reduces crop yield and productivity (Onuh *et al.*, 2015; Adigun *et al.*, 2020).

Weeds are undesirable plants that compete with crops in agricultural fields for essential resources such as water, nutrients, light, and space. Consequently, crops like cowpea exhibit reduced growth and yield in the presence of weeds. The first 4 to 6 weeks after crop emergence are crucial, as weed growth during this period can reduce yields by 50 to 80% (Chikoye *et al.*, 2014). Yield loss depends on weed species, density, environment, and interference duration (Knezevic *et al.*, 2019; Adigun *et al.*, 2023). Effective weed management in crop fields is crucial for enhancing yields and safeguarding both crops and soil. While chemical control remains the predominant method, various alternative strategies are also available. Chemical weed control has a clear edge over traditional methods, including rapid action, cost-efficiency, labor savings, and consistency (Powles & Yu, 2010; Heap & Duke, 2018). However, these methods may cause environmental harm, as their persistence can contaminate soil and groundwater and disrupt the food chain by entering plants as non-target organic contaminants (Albuquerque *et al.*, 2021; Riyaz *et al.*, 2022; Kudsk, 2025).

Sulfonylureas have attained global prominence among herbicide chemistries since their introduction in the 1980s, primarily due to their high herbicidal activity at low application rates (typically 2–40 g ha⁻¹), broad-spectrum weed control, favorable environmental characteristics, and low acute mammalian toxicity (Brown, 1990; Sondhia, 2025). Metsulfuron-methyl (MSM), a systemic sulfonylurea herbicide, is widely used for both pre- and post-emergence management of annual and perennial broadleaf weeds in cereal crops, grasslands, forestry plantations, and non-crop areas worldwide (Idziak *et al.*, 2023). After foliar and root uptake, MSM is rapidly translocated through xylem and phloem to meristematic tissues, where it inhibits acetolactate synthase (ALS), also known as acetohydroxyacid synthase (Tranel & Wright, 2002; Yu & Powles, 2014). ALS catalyzes the first step in producing branched-chain amino acids (valine, leucine, and isoleucine). Inhibiting ALS depletes these amino acids, halting protein synthesis, stopping cell division, and causing plants to cease growing and die (Duggleby & Pang, 2000; Powles & Yu, 2010).

MSM persists in soils for 7 to 60 days, depending on factors such as pH, organic matter, moisture, temperature, and microbes (Maznah *et al.*, 2022). In alkaline soils (pH > 7.0), herbicides can remain active for months, risking carry-over injury in crop rotations, especially affecting sensitive legumes after cereals (Brown, 1990; Kudsk, 2025). Even low residual levels (0.1–1.0 µg kg⁻¹ soil) can impair germination, root growth, photosynthesis, nodulation, and biomass, harming crop establishment and yield (Albuquerque *et al.*, 2021; Riyaz *et al.*, 2022; Bamel *et al.*, 2024). Herbicide spraying may expose adjacent plants to these chemicals, potentially causing injury and morphological abnormalities (Riter *et al.*, 2021; Riyaz *et al.*, 2022). Legume species are more susceptible to herbicides during early growth stages because ongoing protein synthesis depends on active cell division at the shoot and root apices (Délye *et al.*, 2020). A robust root

system in cowpea enables efficient water uptake, root hair infection, nodule formation, and nitrogen fixation. Together, these processes enhance plant growth and productivity (Koech *et al.*, 2021; Peoples *et al.*, 2021). Herbicides have been reported to impair root development, leading to decreased nutrient absorption, limited leaf expansion, reduced photosynthetic activity, and lower seed yields (Singh & Singh, 2021). Although several studies exist on weed control and ALS resistance, only limited data are available that discuss cowpea's response to MSM. If we consider MSM's effects on germination, biomass, and pigment metabolism, substantial research exists on these topics (Barros *et al.*, 2022; Idziak *et al.*, 2023). Most existing studies focus on herbicide efficacy against weeds or on resistance mechanisms. In contrast, relatively few examine herbicide effects on early morphological and biochemical responses in pulse crops (Powles & Yu, 2010; Barros *et al.*, 2022). A comprehensive understanding of these responses is essential for developing effective herbicide management strategies, optimizing application rates and timing, minimizing crop injury, and improving the sustainability of legume-based systems (Kudsk, 2025). This study evaluated the phytotoxic effects of metsulfuron-methyl (MSM) on cowpea (*V. unguiculata*) during early seedling development by examining four herbicide concentrations and their impact on seed germination, growth, biomass, leaf development, and chlorophyll content. The hypothesis is that increasing MSM concentrations will reduce germination, growth, and chlorophyll content in a dose-dependent manner by disrupting physiological processes. This study aims to determine harmful MSM thresholds for cowpea, evaluate MSM phytotoxicity, and develop strategies to minimize crop damage while supporting sustainable legume cultivation.

2. Materials and Methods

2.1. Seed Collection and Sowing

For this study, we selected healthy, uniform cowpea (*V. unguiculata*) seeds from a local seed market in Azamgarh, Uttar Pradesh, India. We excluded unhealthy and damaged seeds to ensure the experiment used only healthy, uniform-size seeds. The seeds were surface-sterilized with 1% (v/v) sodium hypochlorite (NaOCl) for 5 min, followed by 4 to 5 rinses with distilled water to remove residual sterilant before sowing.

2.2. Herbicides Details and Solution Preparation

A fertilizer supplier in Azamgarh provided a commercial MSM product. We prepared a stock solution at 300 mg L⁻¹ by dissolving the herbicide in distilled water. We prepared subsequent dilutions from the stock solution using the formula $C_1V_1 = C_2V_2$. Control plants received distilled water only, without herbicide.

2.3. Experimental Design and Treatment Pattern

This study included two experiments: a laboratory germination test and a disposable cup experiment. During the germination test, multiple metsulfuron-methyl (MSM) solutions were applied to Petri dishes for two days. In the disposable cup experiment, cowpea plants received foliar sprays with varying MSM concentrations. Both experiments utilized a randomized block design with four treatments. Distilled water served as the control, while the second treatment consisted of 100 L⁻¹ metsulfuron-methyl. The third and fourth groups received MSM at concentrations of 200 mg L⁻¹ and 300 mg L⁻¹, respectively. Each treatment included three biological replicates, and we collected observations independently for each replicate.

2.4. Parameters studied

2.4.1. Seed Germination

We evaluated seed germination under controlled laboratory conditions using the Petri dish method. We placed surface-sterilized seeds in sterile Petri dishes lined with two layers of Whatman No. 1 filter paper. Each dish contained 10 seeds, and the filter paper was moistened with either distilled water (control) or the appropriate metsulfuron-methyl solution. We maintained Petri dishes at $25 \pm 2^\circ\text{C}$ and moistened them with treatment solutions. We assessed germination daily until no additional seeds germinated. Seeds were classified as germinated when the radicle extended approximately 2 mm beyond the seed coat. After the 7th day, germination percentage was calculated using the following formula:

$$\text{Germination Percentage} = \frac{\text{Number of germinated seeds}}{\text{Total Number of Seeds}} \times 100$$

2.4.2. Experimental Process for Foliar Application

The experiment took place outdoors at Shibli National College, Azamgarh, India. Plastic cups measuring 25 x 25 cm were filled with a growth medium composed of garden soil, river sand, and farmyard manure in a 2:1:1 ratio. This study used sandy loam soil. We sowed five seeds per cup at a depth of approximately 2 cm. After germination, we thinned seedlings to retain three healthy, uniform plants per cup. We arranged cups randomly and repositioned them periodically to minimize environmental variation. MSM was applied at the three-leaf stage, 15 days after planting, at 5 mL per plant to ensure full leaf coverage without runoff. Control plants received an equivalent volume of water. All plants were watered regularly and maintained under consistent environmental conditions.

2.4.3. Growth Parameters

At 30 days after sowing (DAS), plants were harvested to assess the different growth parameters such as plant height, plant biomass, number of leaves per plant, and leaf area. Plants were carefully extracted from each treated pot, ensuring that soil remained on the roots to minimize damage. Residual soil was gently removed, and roots were washed under running tap water. Root and shoot lengths were measured using a meter scale. Following washing, plants were blotted with sheets to remove excess water. Roots and shoots were separated and weighed using an electronic balance to determine fresh mass. Samples were subsequently dried in an oven at 70°C for 48 hrs to measure dry mass. The number of leaves on treated plants was determined manually. To ensure accuracy, only fully expanded, uniform-sized leaves were included in the count. A fully expanded leaf was chosen for area measurement using the graph paper method. Results are reported in cm^2 .

2.4.4. Biochemical Parameters (Estimation of Chlorophyll Content)

Chlorophyll content was determined according to (Arnon, 1949). For this, 100 mg of fresh leaf samples were crushed in 80% acetone, and the homogenate was centrifuged at 5,000g for 10 min to extract pigments. Chlorophyll concentration was measured in the supernatant using a spectrophotometer, and absorbance was recorded at 663 nm and 645 nm. Results were expressed in mg g^{-1} fresh weight (FW).

2.5. Statistical Analysis

The experiment had three independent biological replicates. Data are shown as mean $\pm$ SE. Statistical analysis was conducted using IBM SPSS Statistics v26.0. We tested treatment means with one-way ANOVA.

We determined differences among treatments using Tukey's HSD ($P \leq 0.05$). Before ANOVA, we checked the data for normality and homogeneity of variance. Means with different lowercase letters differed significantly by Tukey's HSD ($P \leq 0.05$).

3. Results

3.1. Effect of MSM on Seed Germination

MSM significantly affected *V. unguiculata* seed germination, with a dose-dependent decline in germination rate (Fig. 1). The control had a rate of $93.00 \pm 0.83\%$, which decreased to $88.00 \pm 0.63\%$ at T_1 (100 mg L^{-1}). Treatments T_2 ($71.00 \pm 0.12\%$) and T_3 ($66.00 \pm 0.15\%$) showed larger reductions, about 5.4%, 23.7%, and 29.0%, respectively. ANOVA and Tukey's test confirmed significant differences among treatments, indicating that higher concentrations reduce seed germination.

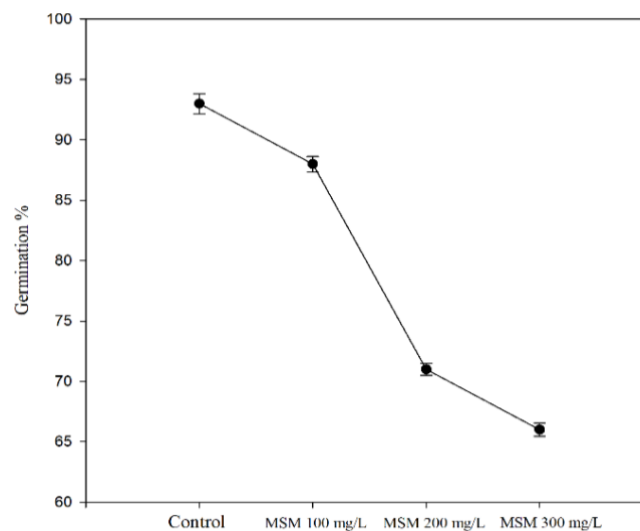


Fig. 1: Effect of metsulfuron-methyl on seed germination of *Vigna unguiculata*.

3.2. Effect of MSM on Seedling Growth and Biomass

The inhibitory effects of MSM intensified during seedling growth (Table 1 & 2). All evaluated growth metrics declined in a concentration-dependent manner compared with the untreated control. Shoot length decreased significantly from $7.50 \pm 0.04 \text{ cm}$ in control seedlings to $5.80 \pm 0.09 \text{ cm}$, $5.40 \pm 0.06 \text{ cm}$, and $3.80 \pm 0.06 \text{ cm}$ under T_1 , T_2 , and T_3 , corresponding to reductions of approximately 22.7%, 28.0%, and 49.3%, respectively. Similarly, root length declined from $3.80 \pm 0.04 \text{ cm}$ in the control to $3.30 \pm 0.06 \text{ cm}$, $3.00 \pm 0.07 \text{ cm}$, and $2.20 \pm 0.06 \text{ cm}$, representing reductions of 13.2%, 21.1%, and 42.1%, respectively (Table 1). These results indicate that root growth was particularly sensitive to increasing herbicide concentrations, with the greatest inhibition observed at the highest treatment level.

Herbicide exposure also reduced fresh biomass accumulation (Table 1). The shoot fresh mass decreased from $16.14 \pm 0.23 \text{ g}$ in the control to $14.27 \pm 0.14 \text{ g}$ and $12.39 \pm 0.20 \text{ g}$ in T_1 and T_2 , representing reductions of 11.6% and 23.2%, respectively. In T_3 , no measurable shoot biomass was present, indicating complete growth inhibition. Similarly, root fresh mass declined significantly from $4.80 \pm 0.13 \text{ g}$ in the control to $3.87 \pm 0.24 \text{ g}$ in T_1 and $2.23 \pm 0.18 \text{ g}$ in T_2 . The plant treated with 300 mg L^{-1} of MSM was nearly dried, so its biomass was not recorded.

Table 1: Effect of metsulfuron-methyl (MSM) on growth attributes of *V. unguiculata* seedlings.

Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh mass (g)	Root fresh mass (g)
Control	7.50 ± 0.04^a	3.80 ± 0.04^a	16.14 ± 0.23^a	4.80 ± 0.13^a

100 mg L ⁻¹	5.80 ± 0.09 ^b	3.30 ± 0.06 ^b	14.27 ± 0.14 ^b	3.87 ± 0.24 ^b
200 mg L ⁻¹	5.40 ± 0.06 ^c	3.00 ± 0.07 ^c	12.39 ± 0.20 ^c	2.23 ± 0.18 ^c
300 mg L ⁻¹	3.80 ± 0.06 ^d	2.20 ± 0.06 ^d	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d

Values are mean ± SE (n = 3). Different superscript letters indicate significant differences via Tukey's HSD test ($P < 0.05$). One-way ANOVA was used. Plants in treatment T₃ showed complete growth inhibition, with zero biomass.

Increasing herbicide concentration severely affected leaf development. Leaf area declined from 9.40 ± 0.11 cm² in the control to 4.30 ± 0.07 cm² and 2.80 ± 0.07 cm² in T₁ and T₂, corresponding to reductions of 54.3% and 70.2%, respectively. In T₃, no measurable leaf area was observed because the seedlings had dried by the time of sampling. The number of leaves per plant also decreased from 7.00 ± 0.08 in control plants to 2.40 ± 0.06 and 2.00 ± 0.07 in T₁ and T₂, representing reductions of 65.7% and 71.4%. T₃ seedlings produced no measurable leaves (Table 2). These findings collectively demonstrate that MSM substantially inhibited seedling growth, biomass accumulation, and leaf development in a concentration-dependent manner.

Table 2: Effect of MSM on leaf development in *V. unguiculata* seedlings.

Treatments	Leaf area (cm ²)	Number of leaves plant ⁻¹
Control	9.40 ± 0.11 ^a	7.00 ± 0.08 ^a
100 mg L ⁻¹	4.30 ± 0.07 ^b	2.40 ± 0.06 ^b
200 mg L ⁻¹	2.80 ± 0.07 ^c	2.00 ± 0.07 ^c
300 mg L ⁻¹	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d

Values are mean ± SE (n = 3). Different superscript letters indicate significant differences via Tukey's HSD test ($P < 0.05$). One-way ANOVA was used. Plants in treatment T₃ showed reduced leaf area and number of leaves per plant.

3.3. Effect of MSM on Chlorophyll Contents

MSM application resulted in a marked reduction of photosynthetic pigment content in *V. unguiculata* seedlings (Table 3). Chlorophyll a level declined from 1.39 ± 0.07 mg g⁻¹ FW in the control to 1.13 ± 0.04 mg g⁻¹ FW under T₁ and 0.64 ± 0.05 mg g⁻¹ FW under T₂, becoming undetectable at T₃. Application of 100 mg L⁻¹ MSM resulted in an 18.7% reduction in chlorophyll content, while 200 mg L⁻¹ MSM led to a 54% decrease. In plants treated with 300 mg L⁻¹ MSM, chlorophyll was not detected due to complete desiccation at the time of measurement. All reductions are reported relative to untreated controls.

Table 3: Impact of MSM on chlorophyll pigment concentrations in *V. unguiculata* seedlings.

Treatments	Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Total chlorophyll (mg g ⁻¹ FW)
Control	1.39 ± 0.07a	0.48 ± 0.05a	1.87 ± 0.04a
100 mg L ⁻¹	1.13 ± 0.04b	0.34 ± 0.04b	1.47 ± 0.03b
200 mg L ⁻¹	0.64 ± 0.05c	0.19 ± 0.04c	0.83 ± 0.03c
300 mg L ⁻¹	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00d

Results are expressed as mean ± standard error (SE), with n = 3. Distinct superscript letters indicate statistically significant differences among treatments according to Tukey's HSD test ($P < 0.05$). Statistical significance was assessed using one-way ANOVA.

Chlorophyll b content decreased from 0.48 ± 0.05 mg g⁻¹ FW in the control group to 0.34 ± 0.04 mg g⁻¹ FW at 100 mg L⁻¹ and 0.19 ± 0.04 mg g⁻¹ FW at 200 mg L⁻¹. At 300 mg L⁻¹ MSM, chlorophyll b was completely depleted. Total chlorophyll content declined from 1.87 ± 0.04 mg g⁻¹ FW in control seedlings to 1.47 ± 0.03 mg g⁻¹ FW at 100 mg L⁻¹ and 0.83 ± 0.03 mg g⁻¹ FW at 200 mg L⁻¹, corresponding to reductions of 21.4% and

55.6%, respectively. Complete pigment loss was observed at 300 mg L⁻¹ of MSM. All results were statistically significant, and chlorophyll pigment decreased in a concentration-dependent manner.

4. Discussion

Pesticides can enter plants in different ways, such as spraying leaves, applying to soil, or treating seeds. This can offer plants both preventative and curative advantages. However, studies indicate that exceeding authorized pesticide application levels can harm plant well-being. Most research on pesticide toxicity currently focuses on its effects on plants (Köseoğlu & Doğru, 2025). This study shows that foliar-applied herbicide MSM induces concentration-dependent phytotoxic effects in cowpea. All parameters, including germination, plant development, and chlorophyll content, decline as MSM concentration increases, with the highest concentration leading to complete plant mortality at the time of sampling (Fig. 1; Tables 1, 2, & 3). These findings align with the established mechanism of sulfonylurea herbicides, which inhibit acetolactate synthase (ALS), the enzyme that initiates biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine (Tranel & Wright, 2002; Green, 2007; Yu & Powles, 2014). These amino acids are essential for protein synthesis, cell division, and normal plant growth. Inhibiting ALS reduces amino acid synthesis, lowers meristematic activity, and impairs plant growth and development (Duggleby & Pang, 2000; Powles & Yu, 2010). The observed reduction in growth parameters and photosynthetic pigment levels with increasing MSM concentrations in cowpea is attributed to disruption of the acetolactate synthase (ALS) system. At the highest tested concentration (300 mg L⁻¹), complete plant mortality was observed, indicating that cowpea is highly sensitive at this exposure level.

Germination begins when a dry seed absorbs water and ends with the emergence of the embryo, typically the radicle, from surrounding tissues. This process concludes at radicle emergence and does not include subsequent seedling growth. During germination, stored food reserves are rapidly mobilized to support seedling development. The results indicate that MSM negatively affects seed germination, with germination rates decreasing significantly as MSM concentration increases (Fig. 1). This reduction may result from the herbicide restricting the mobilization of reserve food materials within the seed (Bewley *et al.*, 2013; Nonogaki, 2019). Reduced seed germination indicates that MSM disrupts critical metabolic processes during seed development by inhibiting amino acid synthesis and key germination enzymes. Amino acids are necessary for protein synthesis, which supports the transition from seed to seedling. (Savage & Bassel, 2016; Buitink & Leprince 2020) have reported the impact of MSM on seed germination. Herbicides are known to inhibit protein synthesis and the activities of key enzymes, such as α -amylase, proteases, and lipases. These enzymes are essential for seed germination, and their inactivation restricts the mobilization of stored reserves necessary for radicle emergence and seedling growth (Wang *et al.*, 2025). These results align with other studies showing that leguminous crops, including soybean, common bean, and chickpea, are vulnerable to ALS-inhibiting herbicides and exhibit reduced seed germination (Albuquerque *et al.*, 2021; Bamal *et al.*, 2024). While some seeds can germinate despite high herbicide concentrations, herbicides such as MSM significantly reduce seedling emergence and inhibit early seed development. As a result, weaker, less vigorous seedlings are produced, which negatively affects field crop performance. Metsulfuron-methyl, in particular, has been shown to harm both seeds and emerging seedlings (Riyaz *et al.*, 2022).

As MSM concentration increases, plant growth characteristics like shoot and root length tend to decrease (Table 1). Interestingly, root length declines more noticeably than shoot length, suggesting that root cells may be more sensitive to the herbicide's effects. The decrease in plant height likely reflects impaired ALS function, as discussed in relation to germination (Délye *et al.*, 2020). Singh & Singh (2021) also reported

poor root growth and disrupted ALS activity in legume crops exposed to the herbicide. Restricted root elongation hinders water and nutrient absorption, limiting growth and performance (Koech *et al.*, 2021; Peoples *et al.*, 2021). Reduced shoot growth impairs the development of photosynthetic tissues, reducing carbohydrate production for roots. When both shoot and root growth are inhibited simultaneously, seedling establishment fails, plant vigor decreases, and susceptibility to stress increases (Parween *et al.*, 2016; Knezevic *et al.*, 2019; Adigun *et al.*, 2023).

Biomass production indicates overall plant productivity. The results demonstrate that MSM application reduces fresh biomass in cowpea, indicating that MSM restricts vegetative growth. Fresh biomass relies on cell division, expansion, and nutrient uptake, all of which depend on protein production and healthy metabolism. When ALS is blocked, plants struggle to produce essential branched-chain amino acids, reducing growth and biomass (Zabalza *et al.*, 2013; Zhao *et al.*, 2018). A 300 mg L⁻¹ MSM concentration caused complete cowpea kill, with no biomass measured (Table 1). Similar biomass reductions at similar levels were reported in other leguminous crops exposed to sulfonylurea herbicides (Bamal *et al.*, 2024). These findings highlight the sensitivity of these species to residual herbicides. They emphasize the importance of proper herbicide application and crop rotation to reduce residue injury and support sustainable farming (Kudsk, 2025).

Leaf initiation and expansion are some of the earliest ways plants respond to herbicide stress. These vital processes rely on cellular activity and protein production in the meristem (Délye *et al.*, 2020). When MSM is applied to cowpea leaves, it notably reduces both leaf number and leaf surface area, and this effect becomes more pronounced at higher concentrations. At 300 mg L⁻¹, complete suppression of leaves was observed (Table 2). These results show that sulfonylurea herbicides block ALS, reducing amino acids, proteins, and cell growth in active plant parts (Yu & Powles, 2014; Tranel & Wright, 2002). The lack of leaf growth at the highest MSM dose indicates it exceeded cowpea seedlings' tolerance, stopping normal leaf formation (Bamal *et al.*, 2024). This concentration harms seedlings and significantly inhibits leaf development. Reduced leaf area affects light absorption and carbon assimilation (Riyaz *et al.*, 2022), and limited canopy growth reduces nutrient production and biomass. Decreased leaf area and chlorophyll suggest damage to the photosynthetic apparatus, further inhibiting growth. These responses diminish cowpea seedlings' ability to grow under increasing herbicide stress (Knezevic *et al.*, 2019; Peoples *et al.*, 2021). Complete death of cowpea at 300 mg L⁻¹ shows that high MSM levels surpass the threshold value of tolerance in cowpea, weakening seedling establishment. The findings demonstrate that sulfonylurea herbicides lack selectivity between weeds and crops, potentially damaging legume species. Therefore, precise application is necessary to reduce the risk to legumes.

Chlorophyll absorbs light and excites electrons during photosynthesis. Higher chlorophyll concentrations improve photosynthetic efficiency. Of the biochemical parameters evaluated, chlorophyll pigments were especially sensitive to MSM exposure. Total chlorophyll dropped from 1.87 ± 0.04 mg g⁻¹ FW to 0.83 ± 0.03 mg g⁻¹ FW at 200 mg L⁻¹, a 56% decline. At 300 mg L⁻¹, it was undetectable due to seedling death. Both chlorophyll a and b decreased with higher herbicide levels, showing that increased MSM harms the photosynthetic system. Reduced chlorophyll indicates impaired chloroplasts and lower photosynthesis, limiting carbon intake and energy for plant growth (Chaudhary *et al.*, 2020). Plants treated with ALS-inhibiting herbicides often show reduced chlorophyll levels, linked to decreased performance under stress (Barros *et al.*, 2021). This decline may result from herbicides disrupting chloroplast function and photosynthesis. Chlorophyll depletion correlates with reduced shoot growth, leaf area, and biomass, indicating decreased photosynthesis in cowpea (Black, 1985). As chlorophyll levels drop with higher

herbicide doses, they could serve as sensitive markers for MSM toxicity and early detection of herbicide stress (Albuquerque *et al.*, 2021; Riyaz *et al.*, 2022).

Cowpea plants exhibit poor growth when exposed to MSM, which disrupts physiological processes essential for normal development. MSM reduces seed germination rates, stunts seedling growth, and lowers leaf chlorophyll content. These effects occur because MSM is structurally similar to phytotoxic chemicals that inhibit the synthesis of essential biomolecules, such as proteins and other critical cellular components (Powles & Yu, 2010; Shah *et al.*, 2022). Higher MSM concentrations increase damage, and high doses can kill seedlings. Even sub-lethal levels significantly restrict growth, posing particular challenges during early development when robust growth is crucial. Our experimental findings have significant implications for cultivating cowpea using MSM for weed management. Cowpea's sensitivity highlights the risk of herbicide carryover in crop rotation systems, particularly when susceptible legume crops are treated with sulfonylurea herbicides. MSM's soil persistence, influenced by environmental variables, may result in residual levels that harm young plants (Brown, 1990). Additionally, spraying can cause phytotoxic effects, underscoring the importance of precise application techniques, appropriate nozzles, favorable weather conditions, and buffer zones to reduce off-target damage (Riter *et al.*, 2021; Riyaz *et al.*, 2022). This investigation evaluates herbicide-induced damage on legume crops. Further research is needed to explore cowpea recovery following sub-lethal herbicide exposure and to examine how environmental factors affect herbicide persistence and toxicity. A deeper understanding of MSM effects can improve herbicide application protocols, ultimately promoting safer weed management practices that align agricultural productivity with environmental sustainability.

4. Conclusion

Herbicides regulate undesired plant growth within agricultural fields; however, their non-target effects are insufficiently explored. Our findings indicate that MSM induces concentration-dependent phytotoxic effects on *V. unguiculata* during early seedling development. MSM exposure markedly reduces seed germination, shoot and root length, plant biomass, leaf area, and chlorophyll concentrations. Complete seedling mortality is observed at 300 mg L⁻¹. These results suggest that cowpea is highly sensitive to MSM, particularly during early developmental stages. The findings highlight the risks associated with residual herbicides and unintended exposure in agricultural systems involving leguminous crops. Effective herbicide management strategies are necessary to protect crops from their toxic effects. This investigation provides empirical evidence on the impact of MSM on cowpea and offers recommendations to improve herbicide management practices and protect vulnerable pulse crops within agricultural systems.

Author Contributions

Supervision, conceptualization and original written draft: A.F.; methodology and review, A; software, AF and N.M.; validation, A; formal analysis, A.F and C.C.; resources, H.K., A.J., R.Z., H.S., S.A, A.B. F.F; data management, H.K., A.J., R.Z; writing - draft preparation, H.K., A.J., R.Z., H.S., S.A, A.B. F.F; All the authors reviewed this manuscript and approved the final version for submission.

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