

Synergistic Effects of Yellow Cosmos (*Cosmos sulphureus* Cav.) Extracts and Adjuvants for Rice Weed Management

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Abstract

This study investigated the weed control efficacy of Yellow Cosmos (*Cosmos sulphureus* Cav.) leaf extract (SNV) in combination with adhesive adjuvants, including sodium lauryl sulphate (SLS), lauryl alkyl sulfonate (LAS), carboxymethyl cellulose (CMC), and esterified vegetable oil (EVO), under greenhouse conditions. Eleven foliar-applied treatments were tested in pot experiments against three dominant rice weeds (i.e. barnyard grass [*Echinochloa crus-galli*], red sprangletop [*Leptochloa chinensis*] and grasslike fimbry [*Fimbristylis miliacea*], grown in mixed culture with the rice cultivar, OM5451.

Our results revealed that SNV combined with SLS or LAS markedly enhanced weed suppression, reducing the height of *L. chinensis* by up to 76.2% and *F. miliacea* by 77.2%, while minimising adverse effects on rice growth (<3.5%). Fresh biomass of *E. crus-galli* declined to 4.8% with SNV+SLS, compared with 6.0% in the SNV alone treatments. These findings highlight that the integration of suitable adjuvants, particularly SLS and LAS, can significantly improve the performance of bioactive plant extracts while maintaining crop safety, offering a promising approach for sustainable rice weed management.

Keywords: Yellow Cosmos, *Cosmos sulphureus* extracts, Adjuvants, Bioherbicide efficacy, Rice weed management, Sustainable agriculture.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops worldwide, which plays a crucial role in ensuring food security and sustaining the livelihoods of millions of smallholder farmers in developing countries (van Dijk et al., 2021; FAO, 2022). However, global rice productivity and production efficiencies are increasingly threatened by various biological constraints, among which weeds feature. Many weeds are primary competitors of rice, particularly in traditional flooded rice farming systems (Chauhan, 2020; Jabran et al., 2021).

Studies have shown that, if not controlled at an early stage, rice-field weeds can cause significant yield losses, ranging from 30% to 60%, while also increasing rice production costs and negatively affecting grain quality (Kumar et al., 2017; Scavo and Mauromicale, 2021).

Herbicides have long been used for weed management in rice for both efficiency and effectiveness purposes. However, the adverse environmental and agronomic effects of excessive herbicide use for managing weeds include the development of herbicide resistance in weeds, the persistence of toxic herbicide residues in soil and water, and other negative environmental impacts on farming environments (Liu et al., 2023; Heap, 2024).

Given those, the search for methods to reduce the reliance on herbicide use in rice and sustainable biological weed management alternatives has gained increasing attention. Among these, plant species with allelopathic activity have been shown to suppress the germination and growth of weeds through the release of secondary metabolites such as flavonoids, phenolic acids and terpenoids (Chauhan and Abugho, 2013; Kong, 2022).

A notable, allelopathic example is Yellow Cosmos (*Cosmos sulphureus* Cav.), a species in the Asteraceae family (Figure 1), which has shown strong inhibitory effects on several major rice weed species, including red sprangletop [*Leptochloa chinensis* (L.)

Nees], lesser fimbry [*Fimbristylis miliacea* (L.) Vahl], and barnyard grass [*Echinochloa crus-galli* (L.) P. Beauv. (Respatie et al., 2019).

To enhance the biological efficacy and stability of *C. sulphureus* extracts, the incorporation of suitable biocompatible adjuvants is considered a crucial strategy. Improving the effectiveness of plant-based extracts through synergistic interactions with adjuvants can increase the surface activity of the formulation, reduce the required amounts of active ingredients and water, and consequently suppress the growth and biomass of target weed species (Turner et al., 1976).



Figure 1. Morphological characteristics of *Cosmos sulphureus*: A. Whole plant; B. Leaves; C. Flowers

Surfactants are a class of compounds capable of reducing surface tension due to their unique molecular structure, which consists of a hydrophobic tail and a hydrophilic head. Based on the nature of the hydrophilic head group, surfactants are commonly classified into four main categories: non-ionic, anionic, cationic and zwitterionic.

Owing to their versatility and structural diversity, surfactants have found wide applications across various fields (Baeurle and Kroener, 2004). Notably, additive or synergistic interactions have been observed in compounds such as atrazine, diuron, hexazinone, simazine and tebuthiuron, which may exhibit cumulative toxicity through the combined biological effects of PS(II) inhibitors in complex mixtures (Magnusson et al., 2010).

The combination of 'Mixture B' and 'Silwet L77' has been shown to enhance the efficacy of imazapyr and metsulfuron-methyl against common

rhododendron (*Rhododendron ponticum* L.) (Lawrie and Clay, 1993). Moreover, sodium lauryl ether sulphate and polyoxyethylene lauryl ether have been reported to disrupt cellular metabolic activity, cause significant membrane damage, and reduce mitochondrial activity and protein synthesis at concentrations ranging from 3.125 μ M to 100 μ M, depending on their synergistic interaction with the herbicide (Song et al., 2012).

Another surfactant, carboxymethyl cellulose (CMC), enhances the adhesion of spray solutions to weed leaf surfaces, thereby prolonging contact time and reducing wash-off caused by rainfall (Yuan et al., 2022; Hao et al., 2020).

Most surfactants, such as sodium lauryl sulphate (SLS) and lauryl alkyl sulfonate (LAS), function by lowering surface tension, promoting deeper penetration into plant tissues and disrupting the cuticular wax layer, thereby facilitating the uptake of

active compounds (Tavares et al., 2019; Liu et al., 2023). In addition, esterified vegetable oils (EVOs), which are chemically modified plant-based oils, not only enhance the solubility of poorly soluble organic compounds but also improve the spreadability and persistence of active ingredients on target surfaces (Meng et al., 2021).

The incorporation of such adjuvants into bioherbicidal formulations holds considerable promise for improving weed control efficacy, while reducing the need for high application rates and minimising unintended effects on crops (Lu et al., 2023; Tataridas et al., 2024). Therefore, the development of plant extract-based bioherbicides combined with suitable adjuvants represents a novel approach to sustainable, safe and environmentally friendly weed management in modern rice production systems.

In this research, our objective was to examine the potential for enhancing the bioherbicidal activity of *Cosmos sulphureus* extracts with a mixture of compatible surfactants, using three significant rice weeds and a rice variety as test plants.

Materials and Methods

Materials

Yellow Cosmos (*C. sulphureus*) leaves for the study were collected at the flowering stage (approximately 60 days after germination) from O Mon District, Can Tho City (Trang et al., 2024). After cleaning and draining, the leaves were chopped into small pieces (1–2 cm) and extracted with methanol. Specifically, 100 g of fresh leaves were soaked in a mixture of 0.6 L methanol and 0.4 L distilled water (6:4, v/v) for 48 hours. The mixture was then filtered through filter paper to obtain the crude extract, which was stored in a refrigerator. The remaining plant residue was further extracted with 0.5 L of cold absolute methanol (100%) for another 48 hours.

During both extraction phases, the mixture was stirred periodically with a glass rod to enhance extraction efficiency. The filtrates from both steps were combined, yielding approximately 1.5 L of total extract. Methanol was subsequently removed using a rotary evaporator (Biobase) under vacuum at 40 °C, resulting in about 200 mL of aqueous extract containing bioactive compounds, following the method described by Thi et al. (2008).

Seeds of the weed species used in the bioassay, i.e. *E. crus-galli*, *L. chinensis* and *F. miliacea*, were collected from rice fields in Nguyen Van Thanh Commune, Binh Tan District, Vinh Long Province. After collection, the seeds were air-dried naturally until their moisture content reached approximately

14–15% and empty or unviable seeds were removed. The rice variety used in the experiment was OM 5451, obtained from the Cuu Long Delta Rice Research Institute (Thoi Lai, Can Tho). All seeds, including both weed and rice seeds, were stored in a refrigerator at 4–5 °C. Prior to the experiments, seeds were subjected to temperature treatment to break dormancy.

The adjuvants used in this study for adhesion and spreading purposes included: lauryl alkyl sulfonate (LAS) 14% (T03 – Neway Company), esterified vegetable oil (EVO) 20.4% (Hasten 70.4SL – Summit Agro), sodium lauryl sulfate (SLS) 14% and carboxymethyl cellulose (CMC) 15%, all of which were supplied by VMC Group. These adjuvants were applied to evaluate their synergistic effects in enhancing the post-emergence weed management efficacy, with concentrations based on the manufacturers' recommendations.

Greenhouse Study Methods

This study builds upon and further refines our previous work (Thi et al., 2024). In the present investigation, the formulation ratios were adjusted by reducing the concentration of *C. sulphureus* extract and increasing the proportion of adjuvants, thereby creating more diverse combinations and enhancing overall treatment efficacy.

The experiment was arranged in a completely randomised block design with 11 treatments, each replicated three times. Each experimental unit consisted of 5 OM 5451 rice seeds and 15 seeds of each weed species, sown in plastic pots measuring 25 × 21 × 21 cm. The experiment was conducted in a stable environment, in a greenhouse, covering 50 m². Treatments were applied by spraying a mixture of *Cosmos sulphureus* extract and adjuvants at a 75:25 ratio, diluted with water to a final volume of 4.69 mL per pot, equivalent to 500 L/ha.

Treatment applications were performed in a controlled-environment spray chamber using a Research Track Sprayer (SB-8, DeVries Manufacturing, Hollandale, MN, USA). The sprayer was equipped with a flat-fan TeeJet 8002E nozzle, operating at a pressure of 275 kPa, and calibrated to deliver a spray volume equivalent to 500 L ha⁻¹. Calibration followed the standard procedure used for laboratory herbicide bioassays. First, the nozzle output (mL min⁻¹) was measured by collecting the spray for 60 s at the operating pressure. This flow rate was then combined with the preset track speed (0.45 m s⁻¹), spray width (0.50 m), and track length (1.52 m) to compute the delivery rate using the standard equation:

$$\text{Spray volume (L ha}^{-1}\text{)} = [\text{Nozzle flow (L min}^{-1}\text{)} \times 600] / [\text{Travel speed (m s}^{-1}\text{)} \times \text{Spray width (m)}]$$

The measured flow and machine speed were adjusted iteratively until the calculated output matched 500 L ha⁻¹. To confirm the calibration, the sprayer was run over the fixed track length while collecting the spray for a known time interval, and the collected volume corresponded to the target delivery per unit area. During all applications, both pressure and travel speed were maintained constant to ensure uniform and reproducible spray deposition. Treatments were applied at their designated dose levels when the weeds reached the 2–3 true-leaf stage.

The following 11 treatments were included: (i) A negative control (distilled water), (ii) *C. sulphureus* extract at 0.24 g/mL, (iii) Esterified Vegetable Oil (EVO) at 20.4%, (iv) Lauryl Alkyl Sulfonate (LAS) at 14%, (v) Sodium Lauryl Sulphate (SLS) at 14%, (vi) Carboxymethyl Cellulose (CMC) at 15%, (vii) *C. sulphureus* extract (0.24 g/mL) + EVO (20.4%), (viii) *C. sulphureus* extract (0.24 g/mL) + LAS (14%), (ix) *C. sulphureus* extract (0.24 g/mL) + SLS (14%), (x) *C. sulphureus* extract (0.24 g/mL) + CMC (15%) and (xi) A positive control (Xevelo 120EC, containing Florpyrauxifen-benzyl 20 g/L + cyhalofop-butyl 100 g/L at 1.25 L/ha (provided by Corteva Agriscience).

Recorded parameters

The biological biomass, including fresh and dry weights of the plants, was analysed at 28 days after treatment (28 DAT). To evaluate the effectiveness of the treatments, efficacy was calculated using Abbott's formula (1925): $H_1 (\%) = [(C_1 - T_1)/C_1] \times 100\%$. In this formula, $H_1 (\%)$ represents the efficacy, C_1 is the height or weight in the control treatment, and T_1 is the height or weight in the treated group.

In addition, to assess the increase in efficacy of sticking agents when combined with the *Cosmos* extracts compared to the use of the extract alone, the following formula was applied: $H_2 (\%) = [(T_2 - C_2)/C_2] \times 100\%$. In this formula, $H_2 (\%)$ indicates the increase in efficacy, C_2 is the height or weight in the treatment with *C. sulphureus* extract alone at a concentration of 0.24 g/mL, and T_2 is the height or weight in the treatment combining the extract with adjuvants or in the positive/negative control treatments.

Statistical analysis

Statistical analyses were conducted in SPSS version 22, with treatment means compared using Duncan's multiple range test ($p < 0.05$).

Results and Discussion

Plant height

The combination of *Cosmos* (SNV) extract with surfactants markedly influenced weed growth. For *Echinochloa crus-galli* (Figure 2), plant height inhibition ranged from 27.2% to 36.6% between 14 and 28 days after treatment (DAT), under the SNV+SLS and SNV+LAS treatments, whereas SNV alone resulted in <10% inhibition. In contrast, EVO and LAS, applied individually, produced only weak inhibition or slight stimulation of growth. CMC exhibited inconsistent effects, including a pronounced growth stimulation at 28 DAT (–57.4%).

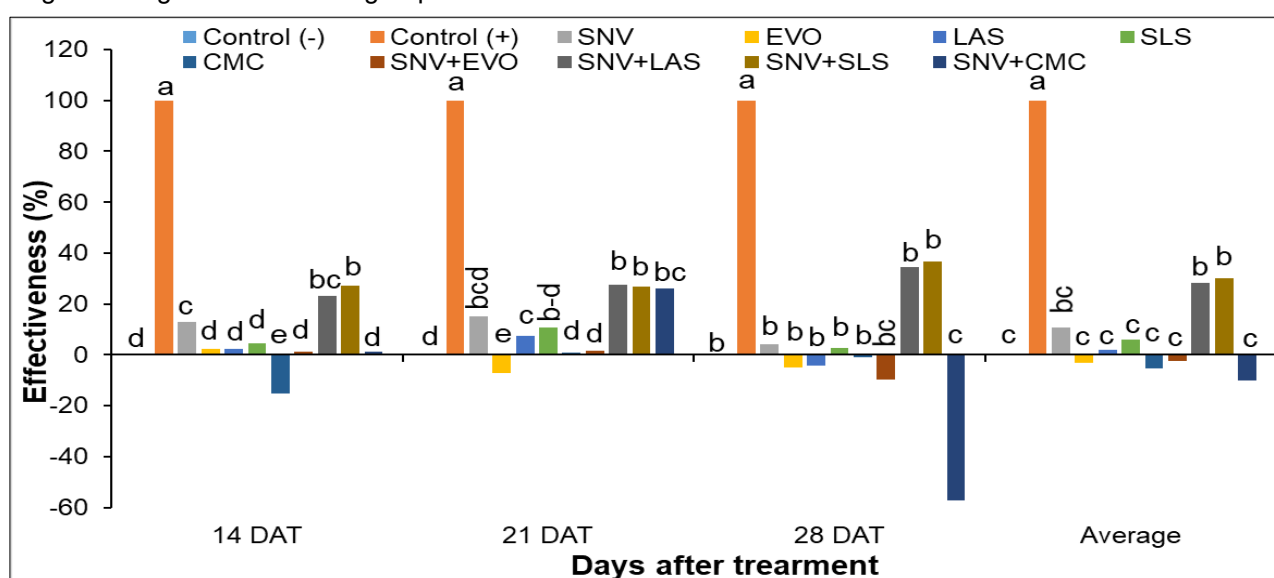


Figure 2 Efficacy of *Cosmos sulphureus* leaf extracts combined with different surfactants on the height of *Echinochloa crus-galli* (Note: Negative values indicate plant-stimulating ability).

On average, across the three assessment dates, SNV+SLS and SNV+LAS caused 30.3% and 31.7% inhibition of plant height of *E. crus-galli*, respectively, confirming their superior performance over SNV alone. These findings align with earlier evidence that surfactants may improve adhesion and uptake of bioactive compounds and, therefore, their bioefficacy (Song et al., 2012; Tavares et al., 2019).

With *Leptochloa chinensis*, SNV alone reduced plant height by 33.8% at 14 DAT, whereas SNV+SLS and SNV+LAS achieved 72.8% and 68.2% inhibition, respectively. Suppression increased further at 21 DAT (80.6% with SNV+SLS, 76% with SNV+LAS) and remained high through 28 DAT (>74%).

The average inhibition of plant height across time points was 76.7% for SNV+SLS and 74% for SNV+LAS. In contrast, treatments with single

adjuvants, such as EVO, LAS, and SLS, produced inconsistent and low efficacy results. Nevertheless, these results highlight the persistence and stability of surfactant-enhanced treatments (Figure 3).

For *Fimbristylis miliacea*, inhibition was only 14.8% with SNV at 14 DAT, but increased to 81.5% and 76.3% with SNV+SLS and SNV+LAS, respectively. High efficacy was maintained at 21 DAT (74.9% and 71.6%) and 28 DAT (75.2% and 71.1%). The mean inhibition levels of 76.1% (SNV+SLS) and 72.1% (SNV+LAS) far exceeded the 30–54% range of other treatments. These findings confirm a strong synergistic effect between SNV and the surfactants, particularly SLS and LAS, in suppressing the growth of *F. miliacea* (Figure 4).

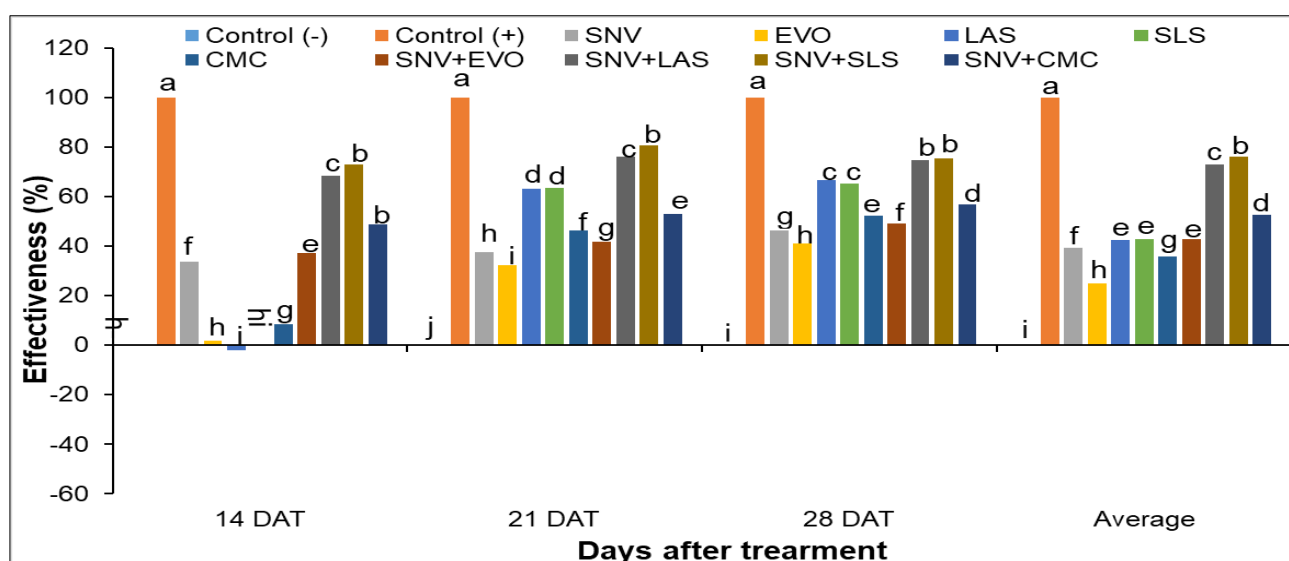


Figure 3. Efficacy of *Cosmos sulphureus* leaf extracts combined with different surfactants on the height of *Leptochloa chinensis* (Negative values indicate plant-stimulating ability).

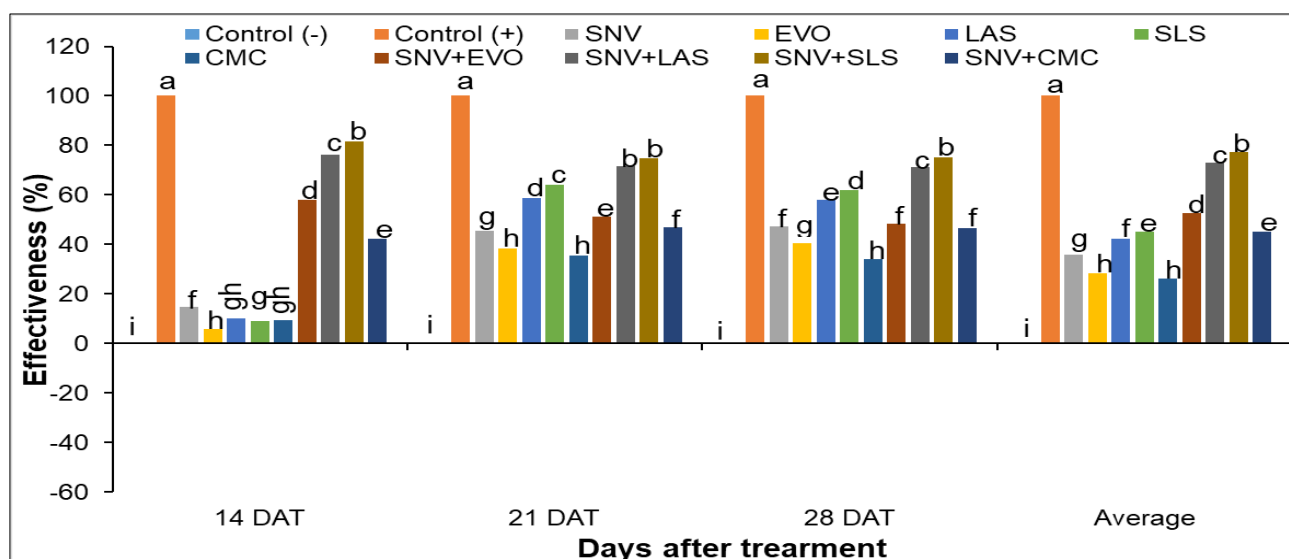


Figure 4. Efficacy of *Cosmos sulphureus* leaf extracts combined with different surfactants on the height of *Fimbristylis miliacea* (Negative values indicate plant-stimulating ability).

Effects on Rice CV OM5451

Measurements of how well the rice cultivar OM5451 responded to the treatments were important in our study. Overall, the rice CV OM5451 showed only minor responses to SNV formulations (Figure 5). At 14 DAT, inhibition was 10.3% for SNV+SLS and about 1% for SNV+LAS, compared with -3.6% stimulation under SNV alone. At 21 DAT, values remained low (1.7% for SNV+SLS, -1.1% for SNV+LAS), and at 28 DAT, they were 1.9% and 2.2%, respectively. Across all assessments, the mean inhibition levels were 3.2% (SNV+SLS) and 1.0% (SNV+LAS), which indicated negligible negative effects. The fresh biomass ratios of rice (1.4–1.5%) were also close to control values. These results, therefore, highlight the selectivity of SNV+SLS and

SNV+LAS treatments: they strongly suppressed the three rice weed species while maintaining crop safety for rice, an essential characteristic required for acceptance of bioherbicides (Hu et al., 2022).

Fresh biomass All Species

The fresh biomass data reinforced these results (Figure 6). For *E. crus-galli*, biomass declined to 4.4% in SNV+SLS and 4.3% in SNV+LAS, compared with 6.0% for SNV alone, 3.3% for SLS, and 3.3% for LAS. For *L. chinensis*, biomass was 5.6% in SNV+SLS and 3.8% in SNV+LAS, markedly lower than 9.3% in SNV alone. Similarly, *F. miliacea* biomass fell to 3.2% (SNV+SLS) and 3.6% (SNV+LAS), compared with 5.2% for SNV alone. These differences were significant ($P < 0.01$).

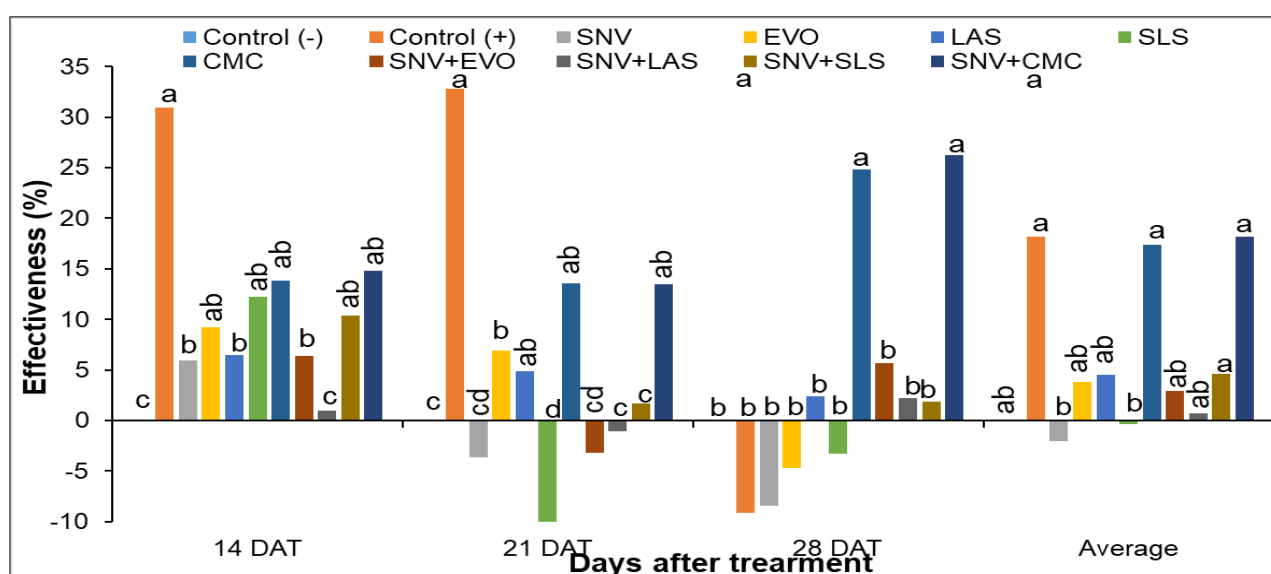


Figure 5 Efficacy of *Cosmos sulphureus* leaf extract combined with different surfactants on the height of OM5451 (Negative values indicate plant-stimulating ability).

The strong reduction in biomass shows that surfactant-assisted formulations outperformed both single components and untreated controls. The synergistic suppression that occurred reflects potentially enhanced adhesion and more uniform distribution of active compounds (Green and Foy, 2004; Liu et al., 2023).

Mechanistic Interpretation

The observed synergistic efficacy can be attributed to the physicochemical properties of surfactants (Figure 7). They are well-known to reduce surface tension and contact angles of droplets, improve the spreading and droplet adhesion, and enhance the penetration of active ingredients of herbicides or bioactive, externally-applied allelochemicals into a treated plant tissue (Mesnage, 2021; Song et al., 2021).

In addition, surfactants, such as SLS and LAS, once they penetrate into a plant, on their own, may act by disrupting cell membranes and reducing mitochondrial function and protein synthesis, thereby enhancing phytotoxicity on treated cells and tissues (Song et al., 2012; Mesnage et al., 2013). The poor performance of EVO and the inconsistent phytotoxicity of CMC underline the importance of selecting appropriate surfactants to support foliar treatments.

Environmental compatibility is also a key advantage that adjuvants provide. For instance, SLS undergoes hydrolysis and subsequent β -oxidation to yield fatty alcohols and inorganic sulphates, while LAS degrades through mineralisation pathways (Bondi et al., 2015; Wang and Keller, 2009). Such biodegradability supports their increased use in sustainable agroecosystems to enhance foliar applications (Figure 8).

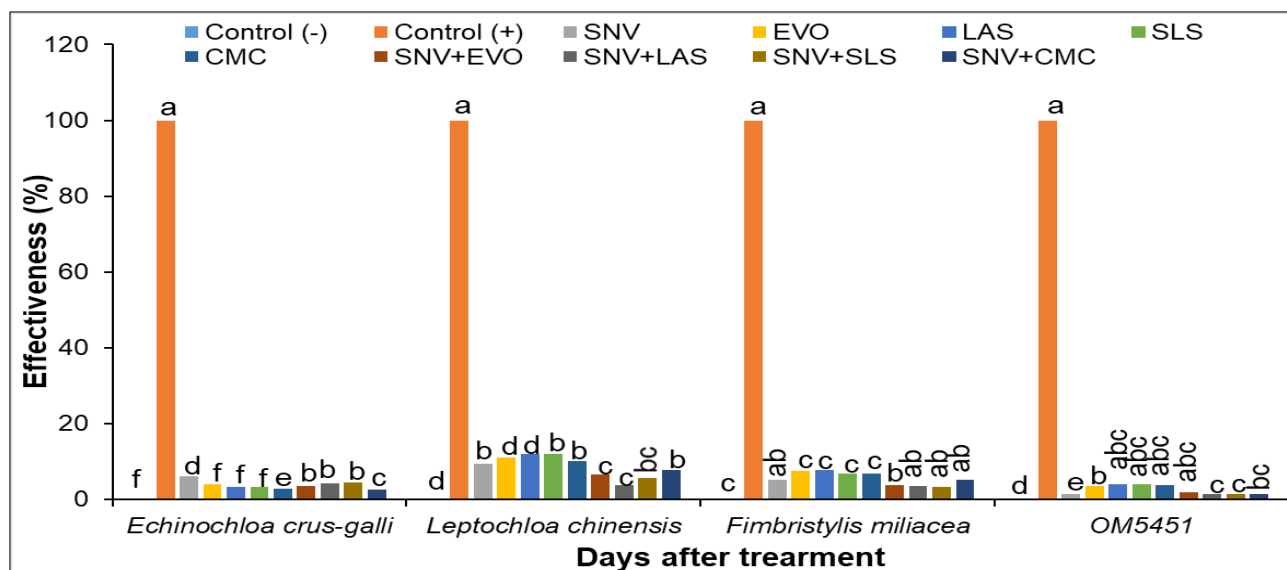


Figure 6. Efficacy of *Cosmos sulphureus* leaf extracts combined with different surfactants on the fresh biomass of the tested plants

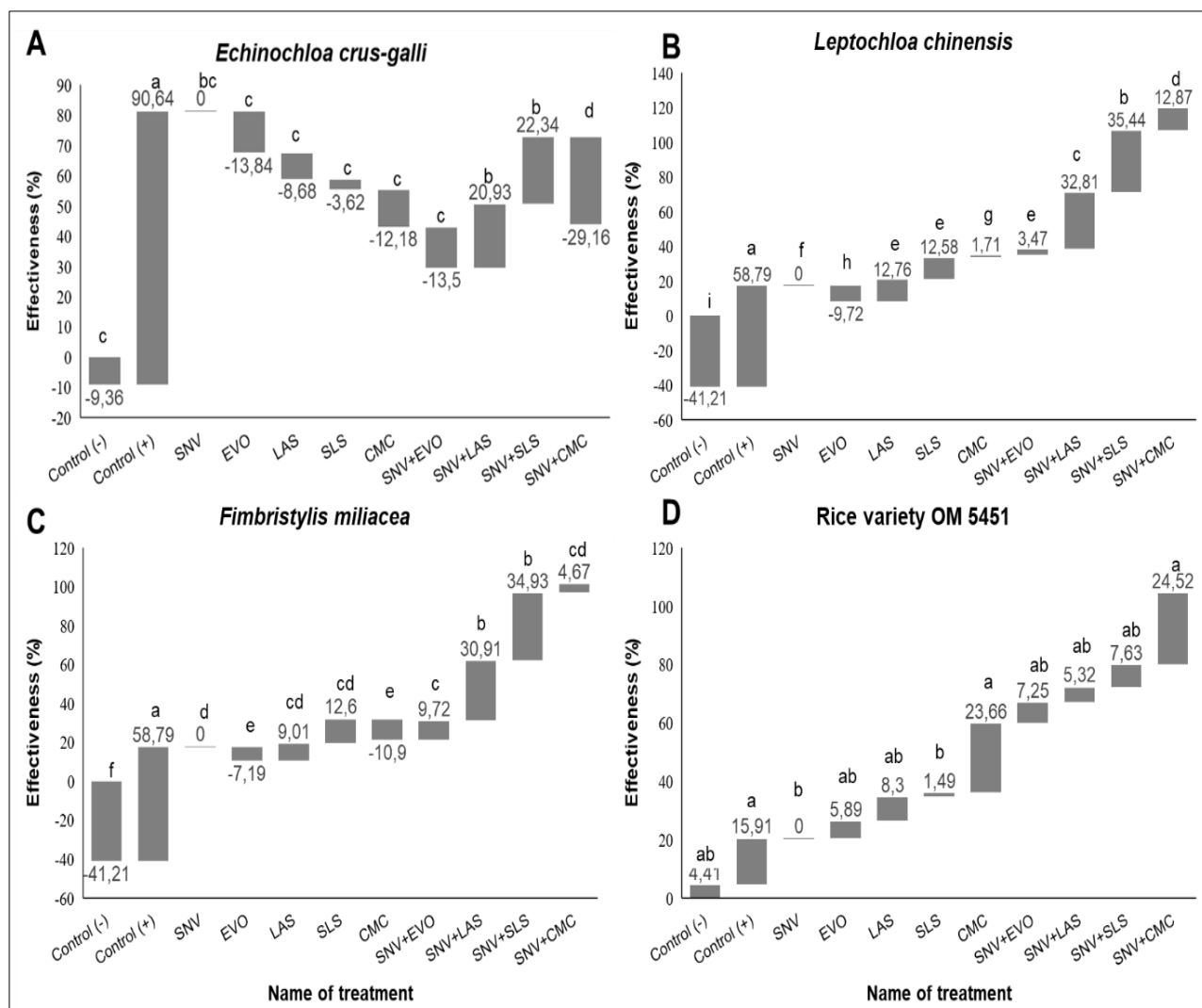


Figure 7 Enhanced efficacy of the surfactants on plant height of test species compared with the sole extract

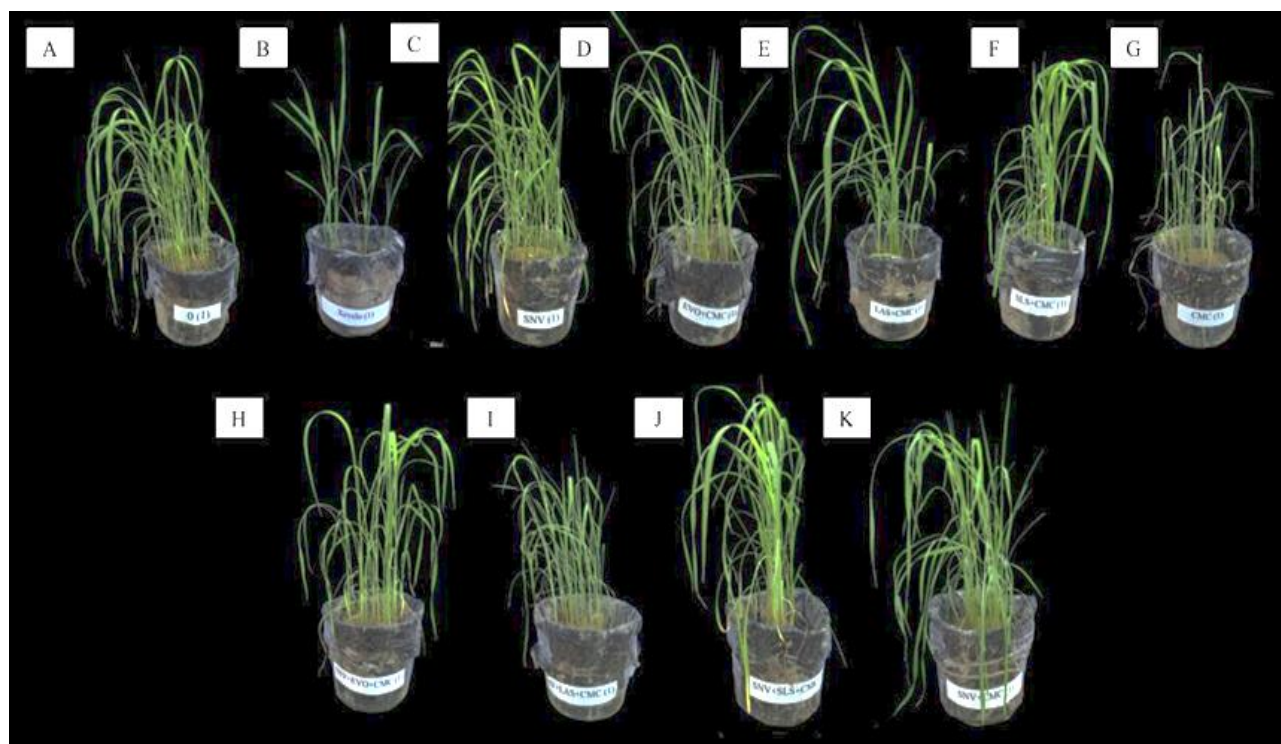


Figure 8 Inhibitory potential of *Cosmos sulphureus* leaf extracts combined with different surfactants at 28 DAT (see Text for details)

(Note: **A**: Control; **B**: Xevelo 120EC; **C**: *Cosmos sulphureus* leaf extract at 0.24 g/ml (Cs); **D**: Esterified Vegetable oil; **E**: Lauryl alkyl sulfonate; **F**: Sodium lauryl sulfate; **G**: Carboxymethyl cellulose; **H**: Cs + Esterified Vegetable oil; **I**: Cs + Lauryl alkyl sulfonate; **J**: Cs + Sodium lauryl sulfate; **K**: Cs + Carboxymethyl cellulose)

Conclusions

The study demonstrated that leaf extracts of *Cosmos sulphureus* (SNV) possess substantial potential to suppress the growth of three major weed species in rice cultivation: *Echinochloa crus-galli*, *Leptochloa chinensis*, and *Fimbristylis miliacea*. When combined with adhesive agents such as sodium lauryl sulphate (SLS) and lauryl alkyl sulfonate (LAS), the herbicidal performance was significantly enhanced.

On average, across the three assessment periods (14, 21, and 28 days after treatment), the SNV + SLS formulation inhibited plant height by 30.15% in *E. crus-galli*, 76.23% in *L. chinensis*, and 77.19% in *F. miliacea*, while also markedly reducing fresh biomass relative to SNV or adjuvants applied individually. Notably, these combined formulations did not negatively affect the OM5451 rice variety and even supported more stable plant growth.

Overall, the results suggest that integrating plant extracts with adjuvants not only improves weed suppression but also maintains ecological safety, underscoring the potential of such combinations in the development of bio-based formulations for

sustainable agriculture. Ongoing studies are currently examining the long-term field performance, optimal application rates, and underlying physiological mechanisms to validate and strengthen these findings, which will further inform the practical deployment of SNV-based bioherbicides in rice production systems.

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