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Allelopathic effects of yellow cosmos (*Cosmos sulphureus* var. *hirsuticaulis*): Phytochemicals composition and its weed suppression potential

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ABSTRACT

We evaluated the allelopathic effects of methanol extracts from different parts of *Cosmos sulphureus var. hirsuticaulis* (CS) on *Brassica juncea* (L.) Czern., used as an indicator species, to compare the inhibitory potential of flowers, leaves, stems and roots. The experiment was done in completely randomized design (CRD) with three replications and 6-extract concentrations (0, 0.03, 0.06, 0.12, 0.24 and 0.48 g/mL). Results showed that the inhibitory effects of CS extracts on germination and growth were concentration-dependent, with stronger suppression at higher concentrations. The flower and leaf extracts exhibited the highest inhibitory potential on root and shoot elongation, significantly stronger than those of stem and root extracts. Phytochemical analysis confirmed the presence of phenolic compounds (64.95 mg GAE/g) and flavonoids (1.96 mg QE/g fresh extract). Among plant parts, flowers contained the highest levels of phenolics (66.32 mg GAE/g) and flavonoids (3.39 mg QE/g). Greenhouse trials further demonstrated that leaf extracts at 0.96 g/mL significantly reduced weed growth, with *Leptochloa chinensis* and *Fimbristylis miliacea* exhibiting 50 % and 48.53 % inhibition, respectively. These findings suggest that CS leaf extracts may serve as an eco-friendly bioherbicide for sustainable weed management in rice production.

Key words: Barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv), red sprangletop (*Leptochloa chinensis* (L.) Nees), grasslike fimbry (*Fimbristylis miliacea* (L.) Vahl), inhibitory effect, yellow cosmos (*Cosmos sulphureus* var. *hirsuticaulis*).

INTRODUCTION

Allelopathy is defined as the biochemical interaction, which can be either beneficial or harmful, between crops and weeds, or between crops and microorganisms. This interaction occurs through the production of chemical compounds (allelochemicals) released into the environment, subsequently affecting the growth and development of neighboring plants. Allelochemicals are present in all plant tissues and are released into the surrounding environment through various mechanisms (decomposition of residues, volatilization and root exudation). These compounds have diverse structures and modes of action and hold the potential to develop ecofriendly bioherbicides in future (25). Specifically, certain plant species in Asteraceae family exhibit the remarkable capacity to produce bioactive compounds that influence the germination and growth of other plants, either by stimulation or inhibition (3,19,24). The Asteraceae family, particularly species like *Acmella oleracea* and *Sphagneticola trilobata*, has demonstrated significant allelopathic potential, offering ecological benefits and promising applications in sustainable agricultural practices (24). Some related secondary metabolites have been identified, including terpenes, saponins, alkaloids, alkamides, cinnamic acid derivatives and flavonoids (17). New discoveries about the enzymes and genes involved in the

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*Correspondence author. [1] Natural Science Faculty, [2] Biotechnology and Food Technology Institute, Can Tho, Vietnam- 900000 production of putative chemical compounds, the presence of chemical compounds in roots, their molecular target sites in sensitive plant species, as well as their effects on other organisms, could lead to the increased use of natural products in pest management, as well as in pharmaceuticals and nutraceuticals (11).

Sesquiterpene lactones play a crucial role in the phytotoxic effects on various plant species, with CS standing out as a notable example. This compound group, widely distributed in the Asteraceae family, is recognized for its significant ecological and biological impact. More than 4,000 sesquiterpene lactones have been identified within this family, representing one of the largest classes of secondary metabolites. Their diverse biological activities, often attributed to the α, β-unsaturated carbonyl group in their lactone rings, have made them a focus of extensive research. In case of CS, these compounds not only contribute to its phytotoxicity but also enhances its ecological adaptability and competitive advantage, demonstrating their importance in the evolutionary success of Asteraceae species (4,26). Over 100 phenolic compounds from six Asteraceae plant species in Siberian Asia, revealing that cultivation of these species can yield bioactive compounds at levels comparable to or exceeding those found in wild samples (3). It indicates the potential for producing new Asteraceae crops rich in phenolic compounds for agricultural use. Extracts from CS flowers show the highest toxicity, followed by leaves, shoots, and roots (21). The inhibitory concentration of allelochemicals on plant species depends on the maturity stage of the plant. Additionally, extracts from CS have shown bioherbicidal activity against weeds without adversely affecting crops (10,21).

Weeds are one of the major obstacles to optimizing productivity in rice production (7). Weeds can reduce rice yields by 50 % to 70 % due to weed competition (10,15). The use of herbicides is common methods to control weeds, however, repeated use of herbicides contributes to increased resistance, environmental pollution and reduced biodiversity (27,31). The impact of weeds on soil biology and ecology in agricultural ecosystems is often overlooked, exacerbating the damage caused by weeds (14). Therefore, it is essential to develop more environmentally friendly and effective weed control measures, such as using allelopathic compounds as bioherbicides (21,22). Researching the weed-suppressive ability of CS extracts in rice fields is considered a valuable tool for the development of effective bioherbicides in future.

MATERIALS AND METHODS

The experiment was conducted in the Biotechnology and Plant Protection Laboratory and a greenhouse at the College of Agriculture, Can Tho University (10.033°N, 105.783°E), altitude 3 m above sea level. The study area has a tropical climate, with an average annual temperature of 27 °C and an annual rainfall 180 cm. The experiment was carried out from November 19, 2023 to April 14, 2024, encompassing both *in-vitro* and *in-vivo* studies under controlled environmental conditions to ensure consistency in data collection.

(I). PETRIPLATE BIOSAAYS

(i). Inhibitory effects of *CS* methanol extract on shoots and roots growth of weeds

Fresh CS samples were collected 60 days after planting, washed thoroughly under tap water, air-dried naturally, and cut into 1-2 cm pieces. A total of 100 g plant material (including roots, shoots, leaves, and flowers) was weighed and placed in an Erlenmeyer flask, then soaked in a methanol:water (6:4) solvent (1 L) for 48 h. The extract was filtered through filter paper, and the remaining residues were re-extracted with 0.5 L of 100 % methanol for 48 h. The two extracts were combined (approximately 1.4 L) and concentrated using a rotary evaporator (Yamato Neocool Circulator CF302L, Yamato Rotary Evaporator RE301, Yamato Water Bath BM510, Yamato. T. Suzuki, Japan) at 40 °C, yielding 200 mL of final extract.

Figure 1. Yellow cosmos (*Cosmos sulphureus* var. *hirsuticaulis*) was collected 60 days after planting at the campus of Can Tho Univeristy (10.033°N, 105.783°E), Can Tho, Vietnam

Figure 2. Seeds of barnyard grass (*Echinochloa crus-galli*), red sprangletop (*Leptochloa chinensis*), grasslike fimbry (*Fimbristylis miliacea*) were collectted in the rice fields at the Cuu Long Delta rice Research Institute, Can Tho, Vietnam

Seeds of barnyard grass (*Echinochloa crus-galli*), red sprangletop (*Leptochloa chinensis*), grasslike fimbry (*Fimbristylis miliacea*) were collected in the rice fields at the Cuu Long Delta rice Research Institute, Can Tho, Vietnam. After harvesting, the seeds were naturally dried to a moisture content of 14-15 % and the empty seeds were removed. Seeds of *Brassica juncea*, a very sensitive species commonly used to test the allelopathic effects of both allelochemicals and plant extracts (2,12), were used as indicator plants in the experiment and were provided by Trang Nong Company (2/35B, Hamlet 2, Vinh Loc B, Binh Chanh, Ho Chi Minh City, Vietnam). Florpyrauxifen-benzyl 20g/l + cyhalofop-butyl 100 g/l was purchased from Dego Agrochemical import-export and Manufacturing Company Limited, Ho Chi Minh City, Vietnam.

The CS extract obtained after evaporation was used for bioassays on the test plant species. The experiment was conducted in a completely randomized design with 3 replicates, consisting of 6-treatments (0, 0.03; 0.06; 0.12; 0.24; 0.48 g/mL of fresh material). Ten pre-germinated seeds of each test plant species were placed in each Petri dish (Ø = 35-50 mm), which was then covered with lids, and sealed with plastic wrap. A micropipette was used to evenly apply the extract at each concentration onto filter paper in each Petri dish. The Petri dishes were then placed in a fume hood at 25 °C until the extract evaporated completely, followed by moistening with 1 mL of Tween 20 solution (0.05 %). The experiment was conducted at a constant temperature of 25 °C in dark.

After 48 h, the shoot and root lengths of the test plants were measured. The inhibitory effect of the CS extract on the roots and shoots of the test plants was calculated according to Abbott (1) through IC_{50} (concentration of extract required for 50 % inhibition) and IC_{90} (concentration of extract required for 90 % inhibition):

$$I (\%) = ((L1 - L2)/L1) \times 100.$$

Where, I: Inhibitory effects of CS extract on the test plants, L1: Length of roots or shoots in control, L2: Length of roots or shoots in treatment.

(ii). Effects of CS extracts (Leaves, Shoots, Roots and Flowers) on *B. juncea*

The experiment was arranged in a completely randomized design with 4 treatments (each representing a different part of CS), each treatment containing 10 test seeds and repeated thrice. Other details are the same with the first experiment.

(II). GREENHOUSE

Effects of *CS* leaf extract on *L. chinensis, E. crus-galli* and *F. miliacea* in greenhouse.

The study was done out from March 14, 2024, to April 14, 2024, under controlled environmental conditions to ensure consistency in data collection. The experiment followed a completely randomized block design (CRBD) with 5-treatments: (i) *C. sulphureus* leaf extract at 0.24 g/mL, (ii) *C. sulphureus* leaf extract at 0.48 g/mL, (iii) *C. sulphureus* leaf extract at 0.96 g/mL, (iv) an untreated control, and (v) Xevelo 120 EC (Florpyrauxifen-benzyl 20 g/L + Cyhalofop-butyl 100 g/L) at the recommended dose (1.25 L/ha) replicated 5 times.

Each experimental unit consisted of pots (30 × 20 × 20 cm) each sown with 4 pre-germinated rice seeds (*Oryza sativa* L., cv. OM 5451), 20 pre-germinated *E. crus-galli* seeds, 20 *L. chinensis* seeds, and 20 *F. miliacea* seeds. OM 5451 ("OM" in OM 5451 stands for O Mon, indicating that the variety was developed in the O Mon district, Can Tho province, Mekong Delta region of Vietnam. These OM rice varieties were bred at the Cuu Long Delta Rice Research Institute (CLRRI) in Vietnam.) The extract treatments were diluted with water to a total volume of 2.65 mL per pot (equivalent to 500 L/ha) and sprayed evenly on the foliage when the rice and weed seedlings reached the 2-3 leaf stage.

For the Xevelo 120 EC treatment, water was introduced 24 h after spraying and maintained at a moderate level for 1-3 days.

To evaluate the inhibitory effects of CS leaf extract, the mortality rates of *L. chinensis, E. crus-galli, F. miliacea,* and OM 5451 rice seedlings were recorded at 6- and 14-days post-treatment. Data from both greenhouse and laboratory experiments were statistically analyzed using one-way ANOVA based on a CRBD model, followed by the Student-Newman-Keuls (SNK) post hoc test to determine significant differences between treatments. This experimental approach allowed for a comprehensive assessment of the bioherbicidal potential of CS leaf extract under controlled greenhouse conditions.

(III). CHEMICAL ANALYSIS

(i). **Qualitative analysis of chemicals in *C. sulphureus* var. *hirsuticaulis* extract**

The remaining CS extract, after being used in the bioassays, was utilized to determine the total phenolic and flavonoid compounds as following.

(ii). **Total phenolics:** The total phenolics content was determined using the method described by (32). The extract was diluted to a concentration of 1 g/mL. A standard curve was established using gallic acid at concentrations of 0.02, 0.04, 0.06, 0.08, 0.10 and 0.12 g/mL and 10 % Folin-Ciocalteu reagent (diluted with water). For the assay, 1 mL of gallic acid solution at each concentration or the CS extract was mixed with 2.5 mL of 10 % Folin-Ciocalteu reagent and allowed to react for 5 min. Afterward, 2 mL of 2 % Na_2CO_3 solution was added. The reaction was carried out at room temperature for 45 min and the absorbance was measured using a spectrophotometer at 765 nm. A standard curve was constructed to determine the total phenolics content in the extract.

(iii). **Total flavonoids:** The total flavonoids content was determined using the method described by (5). The extract was diluted with methanol to a concentration of 1 g/mL. A quercetin standard curve was constructed using concentrations of 0.02, 0.04, 0.06, 0.08, and 0.1 g/mL in water. Solutions of 10 % $AlCl_3$ and 1M CH_3COOK were prepared and diluted with water. For the assay, 0.5 mL of quercetin solution at each concentration or the extract was mixed with 1.5 mL of methanol and allowed to react for 5 min. Then, 0.1 mL of $AlCl_3$ was added, and the reaction proceeded for 6 min. Finally, 0.1 mL of 1M CH_3COOK and 2.8 mL of distilled water were added to each mixture, shaken for 1 min and allowed to stabilize at room temperature for 45 min. After stabilization, the absorbance was measured at 510 nm using a spectrophotometer. A standard curve was constructed to determine the total flavonoids content in the extract.

RESULTS AND DISCUSSION

(I). PETRI PLATE BIOASSAYS

(i). Inhibitory effects of CS methanol extract on shoots and roots growth of weeds

The root growth of indicator species *Brassica juncea* was increasingly inhibited as the extract concentration increased. At the lowest concentration of 0.03 g/mL, root growth was inhibited by 18.75 %. This value increased substantially to 39.68 % at 0.06 g/mL and further to 91.19 % at 0.12 g/mL. Root growth was completely inhibited at both 0.24 g/mL and 0.48 g/mL, indicating that the optimal concentration for root inhibition by CS extract was at or above 0.24 g/mL. This suggests that *B. juncea* is highly responsive to the CS extract, with maximal root growth inhibition achieved at intermediate to high concentrations (Figure 3 and 5).

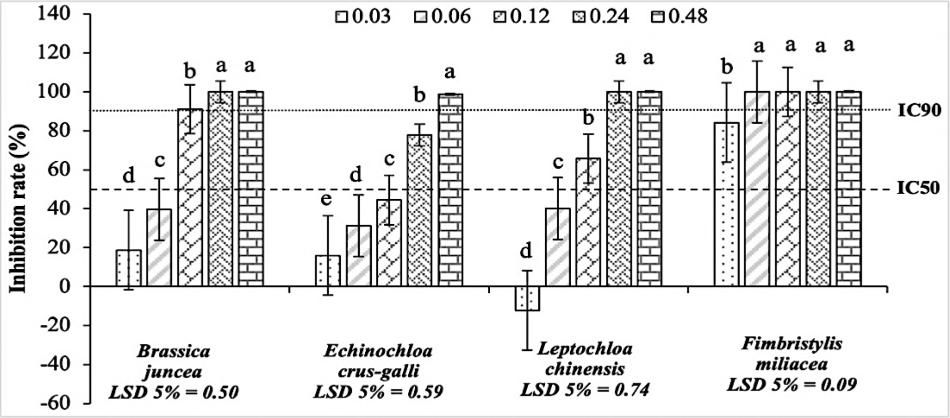

Figure 3. Effects of *C. sulphureus* total extracts at different concentrations (0.03; 0.06; 0.12; 0.24; 0.48 g/mL of fresh material) on root length of weeds [(Differences between treatments were indicated by different letters (a, b, c, etc.), as determined by the Student-Newman-Keuls (SNK) test. Treatments sharing the same letter are not significantly different from each other ($p < 0.05$)].

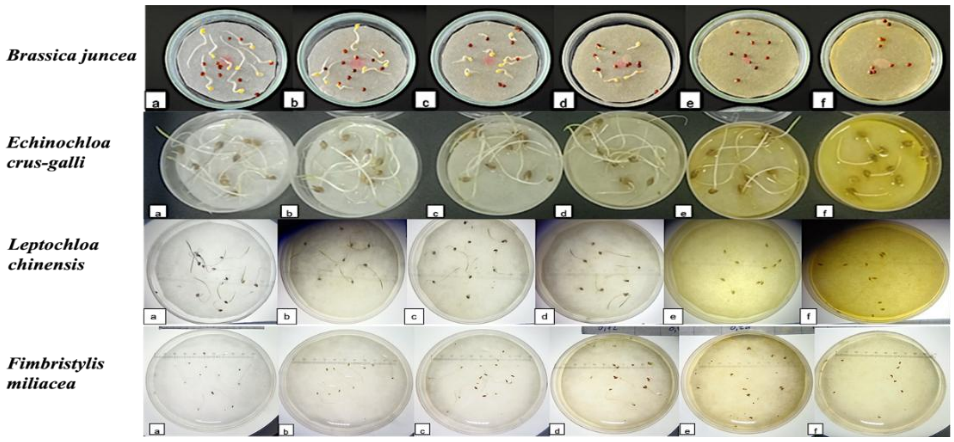

Figure 5. Effects of *C. sulphureus* total extracts at different concentrations (a: 0, b: 0.03; c: 0.06; d: 0.12; e: 0.24; f: 0.48 g/mL of fresh material) on shoots and roots growth of weeds after 48 h

For *E. crus-galli*, a similar trend of increasing root growth inhibition was observed. The inhibition of root growth was 15.91 % at 0.03 g/mL, with the inhibition progressively increased up to 98.74 % at 0.48 g/mL. *L. chinensis* exhibited a unique response pattern, with the root growth being initially stimulated by 12.2 % at the lowest concentration of 0.03 g/mL. However, as the concentration increased, the root growth was inhibited significantly, reaching 40.08 % at 0.06 g/mL and 65.72 % at 0.12 g/mL. Root growth was completely inhibited at concentrations over 0.24 g/mL. In contrast, the root growth of *F. miliacea* was highly sensitive to CS extract even at lower concentrations. The inhibition ranges from 84.16 % at 0.03 g/mL to 100 % at the concentrations over 0.06 g/mL (Figures 3 and 5).

These results provide insights into the differential effects of the CS extract on root development across various plant species, highlighting both the responsiveness and tolerance to varied concentrations.

The CS methanolic extracts significantly inhibited root growth of the test species *L. chinensis*, *E. crus-galli*, *F. miliacea*, and *B. juncea*. Most species exhibited an IC_{50} value around 0.12 g/mL, except for *E. crus-galli*. Among the tested species, *F. miliacea* was the most sensitive with $IC_{50} < 0.12$ g/mL, followed by *L. chinensis* (0.12 g/mL), *B. juncea* (0.12 g/mL), and *E. crus-galli* (>0.12 g/mL). The IC_{90} values ranged from 0.03–0.06 g/mL for *F. miliacea*, 0.12–0.24 g/mL for *L. chinensis*, 0.12 g/mL for *B. juncea*, and >0.24 g/mL for *E. crus-galli* (Figures 3 and 5).

The results demonstrate that various concentrations of CS extracts have differing impacts on weed germination and growth under laboratory conditions, with a clear concentration-dependent effect. Figure 3 showed that at lower concentrations (0.03-0.12 g/mL), the CS extract had a modest impact on root length. However, as the concentration increased to 0.24 and 0.48 g/mL, there was a significant decline in root growth, indicating stronger phytotoxic effects at higher concentrations. This trend aligns with previous studies showing that plant extracts, from the Asteraceae family, contain phenolic compounds and flavonoids which inhibit seed germination and root elongation (3,4,26).

These reductions in root length are likely due to secondary metabolites in the extracts, which interferes with cellular processes such as auxin transport, cell division and nutrients absorption (16,30). The significant root growth inhibition observed in *F. miliacea*, *E. crus-galli* and *L. chinensis* suggests that CS extract could serve as a natural herbicide, potentially controlling weed populations by disrupting root development (9,23).

Figures 4 and 5 illustrates the substantial differences in the inhibition of shoot growth across the test species by varying concentrations of CS extracts. In *B. juncea*, the shoot growth was initially stimulated by 40.62 % at the lowest concentration of 0.03 g/mL but the growth was completely inhibited at 0.24 g/mL. For *E. crus-galli*, the shoot growth was also promoted at the two lower concentrations (0.03 and 0.06 g/mL) by 15.21 %, while the growth was inhibited at the higher concentrations up to 77.24 % at 0.48 g/mL with inhibition surpassing the IC_{50} level at 0.48 g/mL. At 0.24 and 0.48 g/mL, both *B. juncea* and *E. crus-galli* experienced inhibition near IC_{90}, significantly reducing shoot growth. For *L. chinensis*, an initial stimulation of 27.43 % was observed at 0.03 g/mL, followed by a sharp increase in inhibition to 99.19 % at 0.48 g/mL. In *F. miliacea*, the inhibition was 12.79 % at 0.03 g/mL, with complete inhibition (100 %) at 0.06 g/mL and higher concentrations (Figures 4 and 5).

In conclusion, the shoot growth of *B. juncea* and *E. crus-galli* was stimulated at 0.03 g/mL, highlighting the pronounced impact on plant growth even at the lowest CS extract concentration. These results differ from the root growth inhibition patterns as concentrations of 0.24 and 0.48 g/mL resulted in shoot inhibition close to IC_{90}.

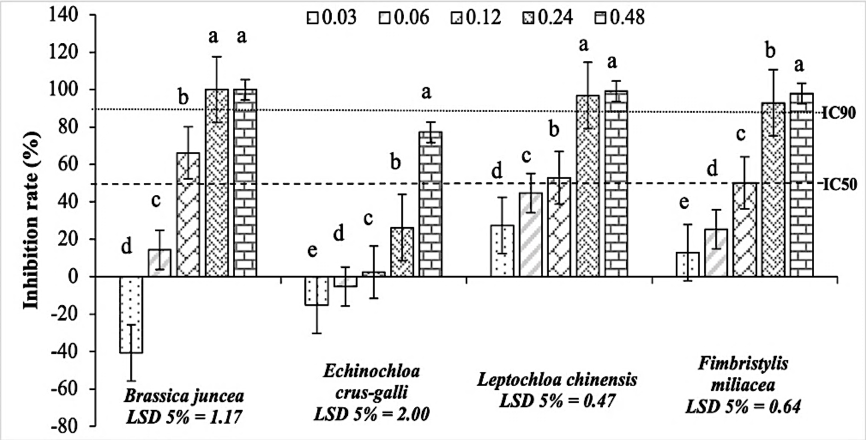

Figure 4. Effects of *C. sulphureus* total extracts at different concentrations (0.03; 0.06; 0.12; 0.24; 0.48 g/mL of fresh material) on shoot length of weeds [(Differences between treatments were indicated by different letters (a, b, c, etc.), as determined by the Student-Newman-Keuls (SNK) test. Treatments sharing the same letter are not significantly different from each other ($p < 0.05$)].

(ii). Inhibitory effects of CS extracts (Leaves, Shoots, Roots and Flowers) on *B. juncea*

The effects of plant part extracts of CS on *B. juncea* root and shoot growth at 48 h exhibited a clear dose-dependent response, with the root growth being more sensitive than the shoot growth. Additionally, shoot elongation showed slight stimulation at lower extract concentrations before transitioning to inhibition at higher concentrations (Figure 6).

For root growth, the inhibitory effects varied depending on the plant part from which the extract was derived. The flower extract exhibited the strongest inhibition, with root elongation reduced by more than 90 % at 0.24 g/mL and nearly 100 % at 0.48 g/mL. The leaf extract followed closely, with inhibition reaching 80 % at 0.24 g/mL and over 95 % at 0.48 g/mL. The stem extract showed a moderate effect and the root extract the weakest inhibition.

Figure 6 illustrates the inhibition rates of different CS extracts on *B. juncea* root elongation. The IC_{50} and IC_{90}values confirmed that root growth was highly sensitive to flower and leaf extracts. The flower extract had the lowest IC_{50} (0.03 g/mL) and IC_{90} (0.06 g/mL), indicating the strongest inhibitory potential. Leaf extracts followed closely, with IC_{50} at 0.06 g/mL and IC_{90} at 0.12 g/mL. Stem extracts had an IC_{50} of 0.12 g/mL and an IC_{90} of 0.24 g/mL, suggesting moderate inhibition. Root extracts were the least effective, requiring 0.24 g/mL to achieve IC_{50} and more than 0.48 g/mL to reach IC_{90}. These results suggest that roots of *B. juncea* were highly sensitive to CS extracts, with extracts from flowers and leaves being the most inhibitory.

Low concentrations (0.03-0.12 g/mL) of CS extract stimulated the shoot growth of *B. juncea*, the allelopathic indicator test plant, while higher concentrations (0.24-0.48 g/mL) significantly inhibited both root and shoot length, consistent with findings from (8).

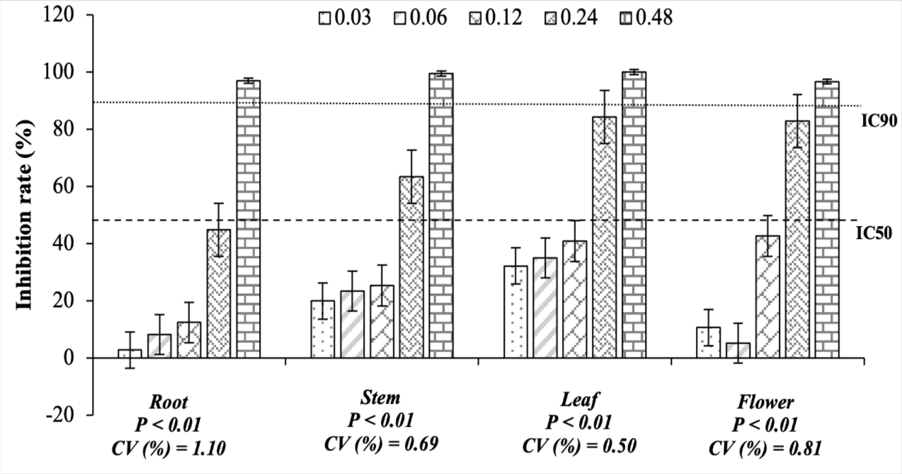

Figure 6. Effects of *C. sulphureus* extracts from plant parts at different concentrations (0.03; 0.06; 0.12; 0.24; 0.48 g/mL of fresh material) on root length of *B. juncea* at 48 h [(Differences between treatments were indicated by different letters (a, b, c, etc.), as determined by the Student-Newman-Keuls (SNK) test. Treatments sharing the same letter are not significantly different from each other ($p < 0.05$)].

In contrast, shoot elongation exhibited a different trend, showing stimulation at lower extract concentrations (0.03-0.06 g/mL), followed by significant inhibition at higher concentrations. At 0.03 g/mL, shoot growth was slightly promoted (5-10 % increase) for all extracts, suggesting a possible hormetic effect at low concentrations (Figure 7). At 0.06 g/mL, shoot elongation remained slightly enhanced (~5 %) for root and stem extracts but showed mild inhibition (~10-20 %) for leaf and flower extracts. At 0.12 g/mL, a clear inhibition trend emerged, with flower and leaf extracts reducing shoot growth by ~50 %, while stem and root extracts inhibiting shoot growth by 20-30 %. At 0.48 g/mL, shoot elongation was nearly completely suppressed (100 % inhibition for flower extracts, 95 % for leaf extracts, 90 % for stem extracts and 75 % for root extracts). Figure 7 presents the inhibition rates of different *C. sulphureus* extracts on *B. juncea* shoot elongation. The IC_{50} and IC_{90} values for shoot inhibition followed the same ranking as root inhibition, but at low concentrations, shoot elongation was initially stimulated before transitioning into inhibition at higher concentrations. The flower extract had the lowest IC_{50} (0.03 g/mL) and IC_{90} (0.06 g/mL), confirming its strong inhibitory effect on shoot elongation. Leaf extracts showed a strong inhibitory effect, with IC_{50} at 0.06 g/mL and IC_{90} at 0.12 g/mL. Stem and root extracts were less inhibitory, with higher values ofIC_{50} and IC_{90} of 0.24 g/mL.

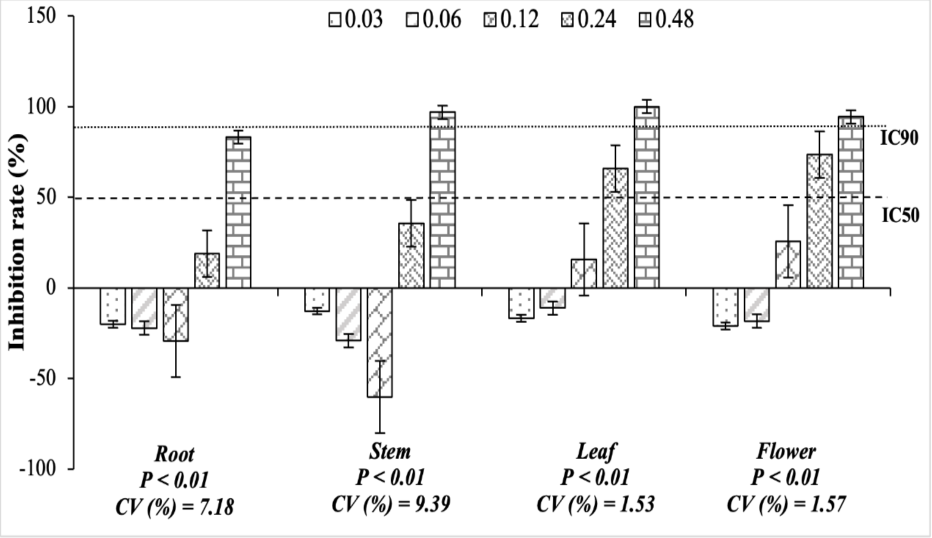

Figure 7. Effects of *C. sulphureus* extracts from plant parts at different concentrations (0.03; 0.06; 0.12; 0.24; 0.48 g/mL of fresh material) on shoot length of *B. juncea* at 48 h [(Differences between treatments were indicated by different letters (a, b, c, etc.), as determined by the Student-Newman-Keuls (SNK) test. Treatments sharing the same letter are not significantly different from each other ($p < 0.05$)]

Overall, the results demonstrated that root growth was more sensitive to *C. sulphureus* extracts than shoot growth. The stimulatory effect of extracts at lower concentrations on shoot elongation suggests a hormetic effect, where low doses may have growth-promoting properties. Among all extracts, flower and leaf extracts exhibited the strongest inhibitory effects on both root and shoot elongation, followed by stem and root extracts. These findings highlight that flower and leaf extracts may contain higher concentrations of allelopathic compounds and could have potential applications as natural herbicides.

Extracts from the flowers and leaves of CS consistently demonstrated superior inhibition effects on plant shoots and roots compared to other parts like shoots and roots (Figure 8). However, practical considerations for collecting material from different parts of CS represent significant challenges. Leaves can be harvested more easily and in larger quantities than flowers, facilitating broader application of the extracts. Therefore, to optimize resource use and application effectiveness, leaf extracts from CS were further assessed on *E. crus-galli*, *L. chinensis*, and *F. miliacea* in the following greenhouse experiment.

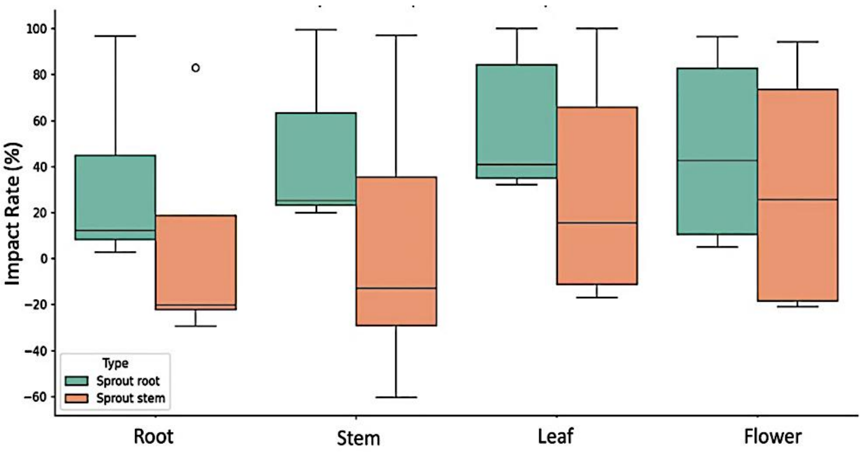

Figure 8. Correlation between different parts extracts of *C. sulphureus* on root and shoot length of *B. juncea* [(Pearson's correlation analysis was used to assess the relationship between varying concentrations of *C. sulphureus* extracts (from leaves, shoots, and flowers) and the root and shoot length of *B. juncea*. Positive or negative correlation coefficients (r) indicate the strength and direction of the relationship, with $p < 0.05$ considered statistically significant)].

(ii) Effects of CS leaf extract on weed growth in greenhouse

Figure 9 illustrates the post-emergent weed control efficiency (%) of *C. sulphureus* leaf extracts at 14 days after treatment on the three weed species. For *E. crus-galli*, the weed control efficiency increased with increasing extract concentration. At 0.24 g/mL, the inhibition rate was approximately 20 %, which was significantly lower than the effects observed at higher concentrations. At 0.96 g/mL, the inhibition rate reached 100 %, which was the highest among the tested extract concentrations. The chemical herbicide treatment also resulted in 100 % inhibition.

For *L. chinensis*, a similar dose-dependent trend was observed, with the control efficiency increasing as the extract concentration increased. At 0.24 g/mL, the inhibition rate was approximately 30 %, followed by 50 % inhibition at 0.48 g/mL and 60 % inhibition at 0.96 g/mL. The chemical herbicide treatment achieved 100 % weed suppression, significantly greater than all extract concentrations.

For *F. miliacea*, the weed control efficiency followed a similar dose-response pattern, but this species was slightly more sensitive to the extracts than the other weeds. At 0.24 g/mL, the inhibition rate was approximately 35 %, with the inhibition increasing to 55 % and 75 % at 0.48 and 0.96 g/mL, respectively.

Overall, the results suggest that higher concentrations of *C. sulphureus* leaf extracts improved post-emergent weed control efficiency. However, the extracts were generally less effective than the chemical herbicide, particularly for *E. crus-galli* and *L. chinensis*, where the chemical treatment significantly outperformed the extracts. Among the three weed species, *F. miliacea* showed the highest sensitivity to the extracts, suggesting that *C. sulphureus* might be more effective against sedge-type weeds compared to grass weeds.

These findings indicate that *C. sulphureus* extracts have potential post-emergent herbicidal properties, but their effectiveness depends on the concentration used and the target weed species. Further studies are needed to optimize application rates and investigate possible synergistic effects with other natural or chemical herbicides (Figure 9).

The strong inhibition of both shoot and root growth at higher concentrations demonstrates that CS extract, especially from flowers and leaves, has potent phytotoxic effects. The IC_{50} levels were generally observed at 0.24 g/mL for both shoot and root growth, where a significant inhibition began, particularly in invasive weeds such as *F. miliacea*, *E. crus-galli* and *L. chinensis*. The study demonstrated the potential of using CS extract as a biological herbicide. Further research into its mechanisms could lead to the development of environmentally sustainable weed management solutions. Additionally, incorporating additives like surfactants could enhance the efficacy of CS extracts in field applications, as seen in other studies focusing on adjuvant-herbicide interactions (13,20). These findings open avenues for developing ecofriendly plant protection methods for weed control and also safe for the environment.

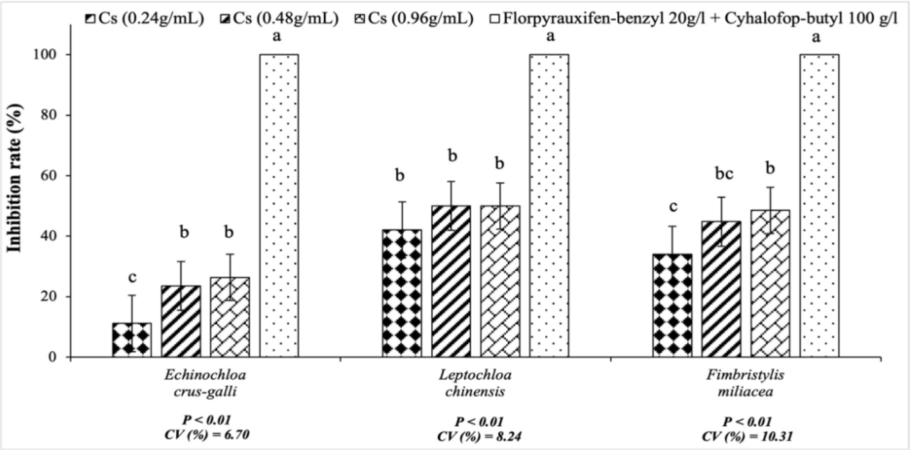

Figure 9. Post-emergence weed control efficiency (%) of *C. sulphureus* leaf extracts and herbicide at 14 d [(Differences between treatments were indicated by different letters (a, b, c, etc.), as determined by the Student-Newman-Keuls (SNK) test. Treatments sharing the same letter are not significantly different from each other ($p < 0.05$)].

(iii) Total phenolics and flavonoids content

The standard curves for phenolics and flavonoids content were established using gallic acid and quercetin as the standards, respectively, with concentration ranges of 0.02, 0.04, 0.06, 0.08 and 0.1 g/mL. The resulting linear regression equations were: $y= 22.919x-5.7317$ for phenolics content and $y = 295.68x-2.4796$ for flavonoids content, with correlation coefficients $R^2=0.9841$ and $R^2=0.9875$, respectively. Based on these standard curves, the total phenolic and total flavonoid content in the *CS* extracts were determined (Table 1).

The total phenolic content varied among different plant parts of *C. sulphureus*, ranging from 64.95 mg GAE/g to 66.32 mg GAE/g in methanol (MeOH) extracts. The flower extract exhibited the highest total phenolic content (66.32 mg GAE/g), which was significantly greater than the composite sample (64.95 mg GAE/g) but not significantly different from the leaf (65.70 mg GAE/g) and stem extracts (65.55 mg GAE/g). The root extract had a total phenolic content of 64.97 mg GAE/g, comparable to the composite sample.

Table 1. Total phenolics and flavonoids in MeOH extracts (1 g/mL) in composite and individual parts of *C. sulphureus*

Extracts	**Total Phenolics** (mg GAE/g fresh extract)	**Total Flavonoids** (mg QE/g fresh extract)
Composite sample of all plant parts	64.9459b	1.9637c
Flower	66.3203a	3.3876a
Leaf	65.6973ab	2.9851b
Stem	65.5470ab	1.8813d
Root	64.9665b	1.7511e

GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent. Values within the same column with different lowercase letters indicate significant differences according to Tukey's test, P = 0.011.

The total flavonoid content showed greater variation, ranging from 1.75 mg QE/g to 3.39 mg QE/g. Among the different plant parts, the flower extract contained the highest flavonoid concentration (3.39 mg QE/g), followed by the leaf extract (2.99 mg QE/g) and the stem extract (1.88 mg QE/g). The root exhibited the lowest flavonoid content (1.75 mg QE/g) among all tested plant parts (Table 1). The composite sample had a flavonoid content of 1.96 mg QE/g, which was lower than the individual flower and leaf extracts but higher than the root extract. Additionally, metabolite analyses revealed that CS extracts, especially from leaves and flowers, had high total phenolics contents (19), which contributed to their strong allelopathic properties. For example, the total phenolics content of whole plant extracts were 64.95 mg GAE/g fresh extract, significantly higher than reported in previous studies, 13.08 mg GAE/g fresh extract (6).

CONCLUSIONS

Methanolic extracts from various parts of *C. sulphureus* exhibited variable inhibitory effects on *B. juncea*, *E. crus-galli*, *L. chinensis* and *F. miliacea*. The degree of inhibition increased with increasing extract concentrations, with the strongest suppression observed at 0.48 g/mL. At this concentration, the extracts inhibited shoot and root growth by an average of 100 %, 87.99 %, 99.59 %, and 98.95 %, respectively. Among the plant parts, extracts from the flowers and leaves consistently showed the highest inhibitory potential, with leaves being favored for practical reasons, such as ease of collection and their abundance. When considering the effects separately, root growth was more sensitive than shoot growth. This suggested that *C. sulphureus* extracts primarily target the root system, leading to greater suppression of seedling establishment. The shoot inhibition also followed a dose-dependent pattern, with lower concentrations showing mild effects and higher concentrations leading to near-complete suppression.

Under greenhouse conditions, treatments with leaf extracts at concentrations of 0.48 g/mL and 0.96 g/mL significantly reduced weed growth at 14 days after application. Specifically, *L. chinensis* experienced 50 % inhibition at both concentrations, and *F. miliacea* exhibited inhibition of 44.8 % and 48.53 %, respectively. These findings highlight that *C. sulphureus var. hirsuticaulis* leaf extracts may be developed as a natural herbicide agent, especially at higher concentrations, to control problematic weed species.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript

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DECLARATIONS

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee

REFERENCES

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18(2):** 265-267.
2. Asghari, J. and Tewari, J. P. (2007). Allelopathic potentials of eight barley cultivars on *Brassica juncea* (L) Czern. and *Setaria viridis* (L) p. Beauv. *Journal of Agricultural Science and Technology* **9(2):** 165-176.
3. Barral Martínez, M., García Oliveira, P., Núñez Estévez, B., Silva, A., Finimundy, T., Calhelha, R., Nenadić, M., Soković, M., Barroso, M. F., Simal-Gandara, J., Ferreira, I., Barros, L. and Prieto, M. (2021). Plants of the family Asteraceae: Evaluation of biological properties and identification of phenolic compounds. *Chemistry Proceedings* **5**: 13. DOI: 10.3390/CSAC2021-10486.
4. Chadwick M., Trewin H., Gawthrop F. and Wagstaff C. (2013). Sesquiterpenoid lactones: Benefits to plants and people. *International Journal of Molecular Sciences* **14:** 12780-12805. DOI: 10.3390/ijms140612780.
5. Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* **10(3)**: 178-182.

6. Chensom, S., Okumura, H. and Mishima, T. (2019). Primary screening of antioxidant activity, total polyphenol content, carotenoid content, and nutritional composition of 13 edible flowers from Japan. *Preventive Nutrition and Food Science* **24(2):** 171.
7. Chin, D.V. (2001). Biology and management of barnyardgrass, red sprangletop and weedy rice. *Weed Biology and Management* **1(1):** 37-41.
8. Gharpure, P., Vaishali, B. and Nivedita, G. (2023). Phytotoxic effects of aqueous leaf extracts of *Cosmos sulphureus* Cav. on *Sorghum bicolor* L., *Vigna aconitifolia* L., *Triticum aestivum* L. *Journal of Tropical Agriculture* **61(2)**: 319-327.
9. Ghayal, N., Biware, M. and Gharpure, P. (2018). Phytotoxic effects of leaf leachates of invasive weeds *Cosmos sulphureus* and *Xanthium strumarium* on agricultural crops. *Biosciences Biotechnology Research Asia* **15(4)**: 821-832.
10. Ho, T.L., Nguyen, C.T., Vu, D.C., Nguyen, T.T., Nguyen, V.Q. and Smeda, R.J. (2021). Rice by-products reduce seed and seedlings survival of *Echinochloa crus-galli*, *Leptochloa chinensis* and *Fymbristylis miliacea*. *Agronomy* **11(4)**: 776.
11. Inderjit, Weston, L.A. and Duke, S.O. (2005). Challenges, achievements and opportunities in allelopathy research. *Journal of Plant Interactions* **1(2)**: 69-81.
12. Kaur, H. and Kaushik, S. (2005). Cellular evidence of allelopathic interference of benzoic acid to mustard (*Brassica juncea* L.) seedling growth. *Plant Physiology and Biochemistry* **43(1)**: 77-81.
13. Kirkwood, R.C. (1993). Use and mode of action of adjuvants for herbicides: A review of some current work. *Pesticide Science* **38(2**): 93-102. DOI: 10.1002/ps.2780380205.
14. Kremer, R.J. (2014). Environmental implications of herbicide resistance: Soil biology and ecology. *Weed Science* ***62*(2**): 415-426. http://www.jstor.org/stable/43700670.
15. Le, D., Chon, N., Kumar, B. and Mann, R. (2018). Weed management practices to control herbicide-resistant *Echinochloa crus-galli* in rice in Mekong Delta, Vietnam. *Research on Crops* **19(1):** 20-27. DOI: 10.5958/2348-7542.2018.00003.7.
16. Li, L., Chen, H., Alotaibi, S.S., Pěnčík, A., Adamowski, M., Novák, O. and Friml, J. (2022). RALF1 peptide triggers biphasic root growth inhibition upstream of auxin biosynthesis. *Proceedings, National Academy of Sciences of the United States of America* **119(31):** e2121058119. DOI: 10.1073/pnas.2121058119.
17. Lopes, A.D., Nunes, M.G.I.F., Francisco, J.P. and dos Santos, E.H. (2022). Potential allelopathic effect of species of the Asteraceae family and its use in agriculture. In: *Vegetation Dynamics, Changing Ecosystems and Human Responsibility,* (Eds., L. Hufnagel & M.A. El-Esawi), pp. 1-15. IntechOpen. DOI: 10.5772/intechopen.108709.
18. Olofsdotter, M., M. Rebulanan, A. Madrid, W. Dali, D. Navarez and D.C. Olk (2002). Why phenolic acids are unlikely primary allelochemicals in rice. *Journal of Chemical Ecology* **28(1):** 229-242.
19. Ortega-Medrano, R.J., Ceja-Torres, L.F., Vázquez-Sánchez, M., Martínez-Ávila, G.C.G. and Medina-Medrano, J.R. (2023). Characterization of *Cosmos sulphureus* Cav. (Asteraceae): Phytochemical screening, antioxidant activity, and chromatography analysis. *Plants* **12(4):** 896. DOI: 10.3390/plants12040896.
20. Polli, E.G., Alves, G.S., Gizotti de Moraes, J. and Kruger, G.R. (2022). Influence of surfactant-humectant adjuvants on physical properties, droplet size, and efficacy of glufosinate formulations. *Agrosystems, Geosciences & Environment* **5(1):** e20230. DOI: 10.1002/agg2.20230.
21. Respatie D., Yudono P., Purwantoro A. and Trisyono Y. (2019). The potential of *Cosmos sulphureus* Cav. extracts as a natural herbicide. *AIP Conference Proceedings* 2202**:** 020077. DOI: 10.1063/1.5141690.
22. Respatie, D., Prapto, Y., Purwantoro, A. and Trisyono, Y.A. (2019). The potential of *Cosmos sulphureus* flower extract as a bioherbicide for *Cyperus rotundus*. *Biodiversity Journal of Biological Diversity* **20(12)**. 3568-3574.
23. Respatie, D.W., Yudono, P., Purwantoro, A. and Trisyono, Y.A. (2021). Effect Spraying volume of *Cosmos sulphureus* Cav. flower extract on weed dominance and soybean yield. In: *IOP Conference Series: Earth and Environmental Science* Vol. **662 (1):** 012017). DOI 10.1088/1755-1315/662/1/012017.
24. Rodrigues, B.N. and Almeida, L.F.R. (2021). Asteraceae family: A review of its allelopathic potential and the case of *Acmella oleracea* and *Sphagneticola trilobata*. *Rodriguésia* **72:** e00292121. DOI: 10.1590/2175-7860202172137.
25. Sangeetha, C. and Bhaskar, P. (2015). Allelopathy in weed management: A critical review. *African Journal of Agricultural Research* **10(9)**: 1004-1015.
26. Schmidt, T.J. (1999). Toxic activities of sesquiterpene lactones: Structural and biochemical aspects. *Current Organic Chemistry* **3**: 577-608. DOI: 10.2174/1385272993377995.
27. Schütte, G., Eckerstorfer, M., Rastelli, V., Reichenbecher, W., Restrepo-Vassalli, S., Ruohonen-Lehto, M., Saucy, A.W**.** and Mertens, M. (2017). Herbicide resistance and biodiversity: Agronomic and environmental

aspects of genetically modified herbicide-resistant plants. *Environmental Sciences Europe* **29(1**): 5. DOI: 10.1186/s12302-016-0100-y. PMCID: PMC5250645.

28. Sofowora, A. (1993). Screening plants for bioactive agents. In: *Medicinal Plants and Traditional Medicine in Africa* (2nd Ed., pp. 134-156). Spectrum Books Ltd, Ibadan, Nigeria.
29. Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. (2011). Phytochemical screening and extraction: A review. *International Pharmaceutica Science* **1(1)**: 98-106.
30. Vanderhoef, L.N., Stahl, C., Siegel, N. and Zeigler, R. (1973). The inhibition by cytokinin of auxin-promoted elongation in excised soybean hypocotyl. *Physiologia Plantarum* **29**(1): 88-92. DOI: 10.1111/j.1399-3054. 1973.tb04804.x.
31. Vencill, W.K., Nichols, R.L., Webster, T.M., Soteres, J.K., Mallory-Smith, C., Burgos, N.R. and McClelland, M.R. (2012). Herbicide resistance: Toward an understanding of resistance development and the impact of herbicide-resistant crops. *Weed Science* **60**(SP1): 2-30. DOI: 10.1614/WS-D-11-00206.1
32. Yadav, R. and Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology* **3(12)**:10-21