Vietnam Journal of Agricultural Sciences

ISSN 2588-1299

VJAS 2025; 8(1): 2359-2372
https://doi.org/10.31817/vjas.2025.8.1.02

# Allelopathic Potential and Hormesis Effect of *Cosmos bipinnatus* Extracts for Weed Management in Rice Cultivation

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## Abstract

Weed management in rice cultivation faces increasing challenges due to herbicide resistance and environmental concerns, necessitating alternative, eco-friendly strategies. Among plant-based bioherbicides, *Cosmos bipinnatus* has emerged as a promising candidate due to its allelopathic potential. This study evaluated the efficacy of *C. bipinnatus* extracts in inhibiting key weed species, namely *Echinochloa zcrus-galli*, *Leptochloa chinensis*, *Fimbristylis miliacea*, and weedy rice (*Oryza sativa f. spontanea*). Bioassays demonstrated significant inhibition of radicle and coleoptile growth in these weeds, with stronger effects at higher concentrations. At 0.48 g $mL^{-1}$, inhibition reached 88% for *E. crus-galli* coleoptiles, 93% for its radicles, and 76% for *L. chinensis* coleoptiles. Notably, weedy rice, a major competitor in rice fields, was effectively suppressed, suggesting the potential of *C. bipinnatus* for integrated weed management. However, low extract concentrations (<0.06 g $mL^{-1}$) induced a hormesis effect, slightly promoting growth in some weeds and rice cultivars (OM380, OM5451, and OM18). Phytochemical analysis identified high phenolic (94.48 mg GAE $g^{-1}$) and flavonoid (514.61 mg QE $g^{-1}$) contents, particularly in leaves (45.93 mg GAE/g phenolic, 107.13 mg QE/g flavonoid) and flowers (63.89 mg GAE/g phenolic, 127.74 mg QE/g flavonoid), indicating their role as key inhibitory agents. These findings highlight *C. bipinnatus* as a viable biological solution for sustainable weed control, particularly against weedy rice. Further research is needed to optimize application strategies and minimize potential crop impacts, ensuring effective field applications while reducing reliance on synthetic herbicides in rice production systems.

## Keywords

Allelopathy, *Cosmos bipinnatus*, flavonoids, phenolics, sustainable weed control

**Received:** December 2, 2024
**Accepted:** March 28, 5

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## Introduction

Allelopathy is a biochemical interaction in which plants release allelochemicals that influence the growth and development of neighboring plant species, either positively or negatively (Cheng & Cheng, 2015). Allelochemicals are organic substances released from various plant tissues directly into the environment through volatilization, leaching, root exudation, or decomposition of plant residues (Zeng *et al.*, 2008). Based on this mechanism, numerous studies have explored the potential of plant-derived extracts or post-harvest residues to control pests in agricultural systems, including crop rotation, insect and disease management, weed control, and invasive species management (Chen *et al.*, 2017; Latif *et al.*, 2017).

Weed infestations can reduce crop yields by up to 34% globally (Jabran *et al.*, 2015). Weeds compete with crops for resources such as light, nutrients, space, and water, leading to decreased crop yield and increased production costs (Ali *et al.*, 2017). Weed seeds can contaminate crop seeds during sowing or germinate from the soil seed bank (Chin, 2001). In rice fields, weeds are particularly prevalent and compete with rice plants, resulting in a 50-70% reduction in rice yield (Ho *et al.*, 2021). Common weed species in Vietnamese rice fields include *Echinochloa crus-galli* (L.) Beauv. (barnyard grass), *Leptochloa chinensis* (L.) Nees. (red sprangletop), *Fimbristylis miliacea* (Vahl) Kunth (grass-like fimbristylis), and *Oryza sativa* f. *spontanea* (weedy rice) (Chin, 2001).

The overuse of synthetic herbicides in agriculture has resulted in environmental degradation, soil nutrient depletion, and the proliferation of herbicide-resistant weed biotypes, posing risks to both crop productivity and human health (Chou, 1999; Montull & Torra, 2023). Allelopathy offers an environmentally friendly alternative to synthetic pesticides for pest management and is a promising source of natural chemicals for agriculture (Farooq *et al.*, 2011; Ain *et al.*, 2023). Integrating allelopathic substances into agricultural management can reduce the use of synthetic chemicals and mitigate environmental degradation (Chou, 1999). Allelopathy can be applied to control weeds in field crops and can play a crucial role in developing sustainable and integrated weed management systems (Macías *et al.*, 2019; Montull & Torra, 2023).

Recent studies have identified members of the Asteraceae family as important sources of allelochemicals with strong weed-inhibitory effects (Lopes *et al.*, 2022). Among them, *Cosmos* spp., commonly grown as ornamental plants, have been reported to contain high levels of bioactive secondary metabolites such as flavonoids and phenolic acids, which are known for their potential role in plant-plant interactions (Hoang & Vo, 2016; Ortega-Medrano *et al.*, 2023). Prior research suggests that extracts from *C. bipinnatus* exhibit phytotoxic effects on certain weed species, but its allelopathic potential in rice agroecosystems remains largely unexplored (Trang *et al.*, 2024).

Natural allelochemicals have been widely used in agricultural weed management, including terpenes, saponins, alkaloids, alkamides, cinnamic acid derivatives, and flavonoids (Lopes *et al*., 2022). However, the allelopathic potential of *C. bipinnatus* has not been fully investigated, making it necessary to evaluate the use of extracts from this species for biological weed control in rice fields. In addition, *C. bipinnatus* is commonly cultivated along rice field borders in Vietnam to attract natural enemies and enhance biodiversity (Nguyen *et al.*, 2015), making it an ideal candidate for sustainable weed management. Its plant residues could serve as an economical and eco-friendly herbicide alternative for suppressing major weed species in rice cultivation.

## Materials and Methods

### Plant materials

Parts of *C. bipinnatus* were harvested from 60 day-old plants on the campus of Can Tho University (CTU), Vietnam. Seeds of *Echinochloa crus-galli*, *Leptochloa chinensis*, and *Fimbristylis miliacea* were obtained from rice fields in Nguyen Van Thanh Commune, Binh Tan District, Vinh Long Province (Syngenta Company's trial farm). Seeds of weedy rice line WR29 were collected from My Hoa Commune, Thap Muoi District, Dong Thap Province and stored at the Biotechnology in Plant Protection laboratory, CTU. After harvest, the seeds were dried to a moisture content of 12-14% and stored in ziplock bags for preservation and use. *Brassica juncea* (green mustard), a highly sensitive species commonly used to test the allelopathic effects of both allelochemicals and plant extracts (Kaur & Kaushik, 2005; Asghari & Tewari, 2007), was used as an indicator plant in the experiment with extracts from *C. bipinnatus* and was supplied by the Trang Nong Company (2/35B, the 2nd hamlet, Vinh Loc B, Binh Chanh, Ho Chi Minh City). Cultivated rice cultivars OM5451, OM380, and OM18, provided by the Cuu Long Delta Rice Research Institute, Vietnam, were selected to evaluate the stimulatory or inhibitory effects of extracts from *C. bipinnatus*.

### Extraction of *Cosmos bipinnatus* extract using MeOH solvent

All fresh samples of *C. bipinnatus* were washed with tap water, blotted dry with clean gauze, and then cut into small pieces (approximately 1-2cm). Fresh samples (100g) were soaked in 0.6L of methanol (MeOH, Kim Nguu Chemical and Equipment Import-Export Joint Stock Company (VietChem), 98%) and 0.4L of distilled water for two days. The mixture was then filtered through filter paper, and the extract was stored in a refrigerator. The remaining residue of the sample was soaked again in 0.5L MeOH (100%) for two days, and the second extract was also filtered similarly. The two extracts were combined to obtain 1.5L of total extract (Le Thi & Kato-Noguchi, 2008). Subsequently, the MeOH solvent was evaporated at 40°C using a rotary vacuum evaporator (Yamato Neocool Circulator CF302L, Yamato Rotary Evaporator RE301, Yamato Water Bath BM510, Yamato. T. Suzuki, Japan), to obtain approximately 200mL of extract containing water and antagonistic substances.

### Evaluation of the *Cosmos bipinnatus* extract allelopathic potential

Using a micropipette, the extract was evenly applied at various concentrations (0.03, 0.06, 0.12, 0.24, and 0.48 from fresh material) onto filter paper placed in 50mm diameter Petri dishes. The Petri dishes g $mL^{-1}$ were then placed in a fume hood (LFS-180S, Yamato Scientific Co., Ltd, Japan) at 25°C and allowed to dry completely (1-1.5 hours), ensuring that all the extract had evaporated. To ensure uniform extract application, the filter papers in the Petri dishes were moistened with 1 mL of 0.05% Tween 20 solution, a non-ionic surfactant that facilitates solubility and distribution of bioactive compounds (Weiszhár *et al.*, 2012).

The experiment was conducted under laboratory conditions in a completely randomized design with three replications, each replication consisted of one Petri dish containing 10 germinated test plant seeds. These Petri dishes were covered and sealed with food wrap and placed in the dark at 25°C. After 48 hours, the lengths of the radicle and shoot of the test plants were measured. The effect of the extract on the test plants was calculated using Abbott's formula (1925): $I (\%) = [(L_1 - L_2)/L_1] \times 100$, where I is the inhibition rate, $L_1$ is the length of the root or shoot of the control plant, and $L_2$ is the length of the root or shoot of the test plant. After 48 hours of dark incubation, the rice was exposed to full light and continuously moistened for the next 120 hours (5 days) to observe any recovery.

### Methods for quantification of total phenolic and flavonoid content

Total phenolic content was determined following the methods of Yadav and Agarwala (2011). The extract was diluted with methanol to a concentration of 1 mg $mL^{-1}$. A standard curve was constructed using gallic acid at concentrations of 0.02, 0.04, 0.06, 0.08, 0.10, and 0.12 mg $mL^{-1}$, mixed with 10% Folin-Ciocalteu reagent. For the assay, 1mL of either the gallic acid standard or the *C. bipinnatus* extract was mixed with 2.5mL of 10% Folin-Ciocalteu reagent and incubated for 5 minutes. Then, 2mL of 2% $Na_2CO_3$ was added, and the reaction was carried out at room temperature for 45 minutes. Absorbance was measured at 765nm using a spectrophotometer. The phenolic content was calculated based on the standard curve and expressed as mg gallic acid equivalent per gram of extract (mg GAE/g extract).

Total flavonoid content was quantified using the aluminum chloride colorimetric method as described by Chang *et al.* (2002). The extract was diluted with methanol to a concentration of 1 mg $mL^{-1}$, and a standard curve was prepared using quercetin solutions at 0.02, 0.04, 0.06, 0.08, and 0.10 mg $mL^{-1}$. A reaction mixture was prepared by adding 0.5mL of either the quercetin standard or the plant extract to 1.5mL methanol and incubating for 5 minutes. Next, 0.1mL of 10% $AlCl_3$ was added and left for 6 minutes, followed by the addition of 0.1mL of 1M $CH_3COOK$ and 2.8mL of distilled water. The mixture was shaken for 1 minute and left at room temperature for 45 minutes. The absorbance was measured at 415nm using a spectrophotometer. The flavonoid content was calculated based on the standard curve and expressed as mg quercetin equivalent per gram of extract (mg QE/g extract).

### Statistical analysis

A one-way ANOVA was used for analyzing the effects of extract concentrations on weed biomass and yield parameters, followed by Duncan's multiple range test ($P$ <0.05) to compare treatment means. For phenolic and flavonoid content comparisons among plant parts, a separate one-way ANOVA was conducted, followed by Tukey's HSD test ($P$ <0.05).

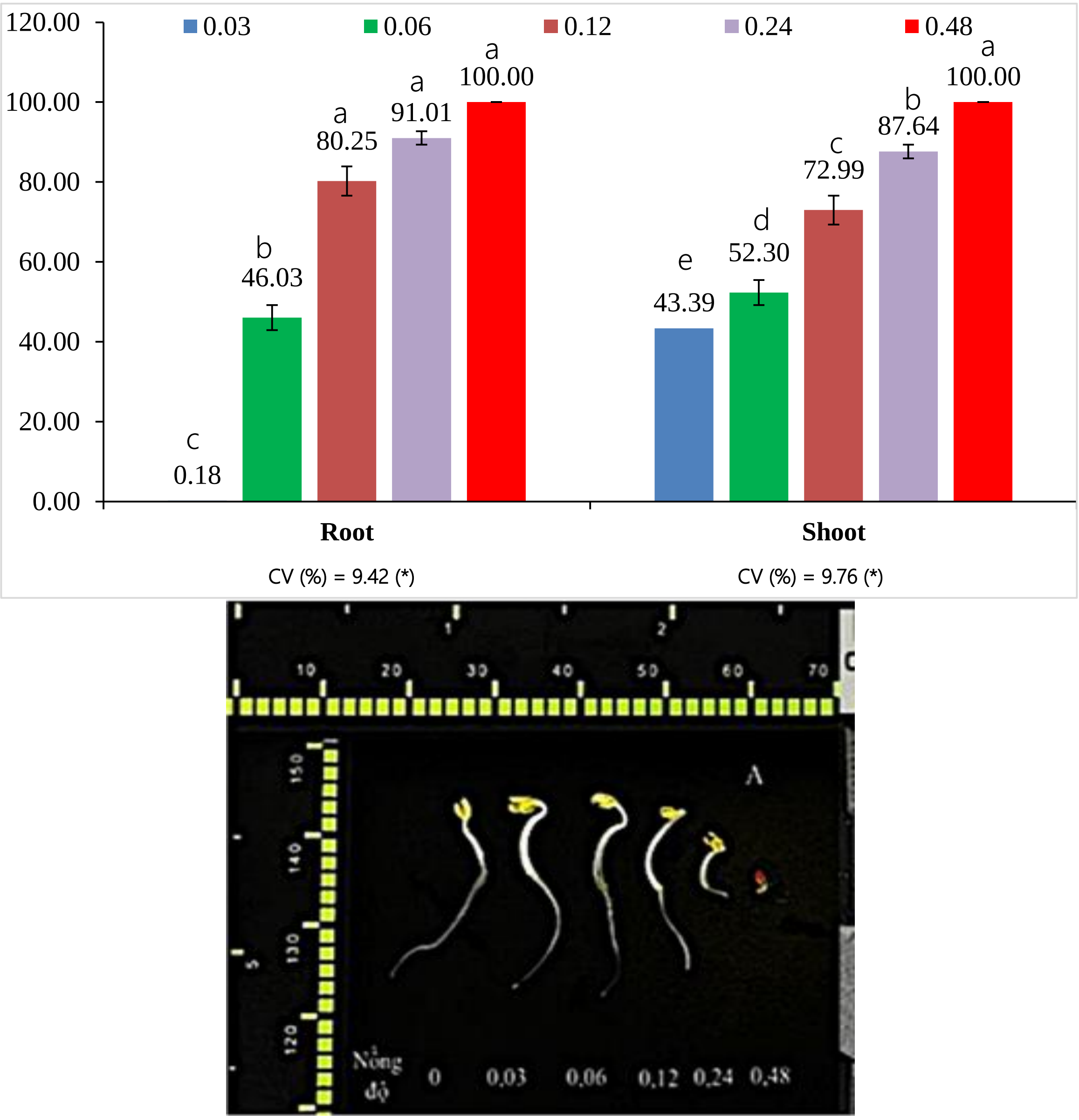
## Results

### Bioactivity of *Cosmos bipinnatus* extract on *Brassica juncea*

**Figure 1** shows that most extract doses from 0.03-0.48 g $mL^{-1}$ had significant inhibitory effects on both the radicles and shoots of *B. juncea* seedlings after 48 hours of testing, with the degree of inhibition increasing with the dose. Specifically, for the radicles, inhibition began at doses of 0.06-0.48 g $mL^{-1}$ (46-100%), and the shoots showed inhibition at all doses, with higher doses resulting in more pronounced inhibition, ranging from 0.03 g $mL^{-1}$(43.4%) to 0.48 g $mL^{-1}$ (100%).

These results indicated that doses ranging from 0.12 to 0.24 g $mL^{-1}$ exhibited potent inhibitory effects, exceeding the 50% inhibitory concentration ($IC_{50}$) in all the tested samples. Moreover, the dose of 0.48 g $mL^{-1}$ consistently maintained a 100% inhibition rate.

The biological activity of the *C. bipinnatus* extracts against various weed species in paddy fields is shown in **Figure 2**. The results indicated that the *C. bipinnatus* extracts at various concentrations exhibited both inhibitory, and in some cases, stimulatory effects on the growth of radicles and coleoptiles of the weed species *E. crus-galli*, *L. chinensis*, *F. miliacea*, and *O. sativa* f. *spontanea*. **Figures 2A and 3** illustrate significant variations in coleoptile inhibition across the different extract concentrations. For *E. crus-galli*, the highest extract concentration achieved up to 88% inhibition of coleoptile growth, while lower concentrations exhibited decreasing inhibitory effects, with the lowest concentration even showing a slight stimulatory effect (3.5%). Similar trends were observed for *L. chinensis*, *F. miliacea*, and *O. sativa* f. *spontanea*, with inhibitory effects decreasing with decreasing concentrations. Notably, *F. miliacea* exhibited a slight stimulatory response (-5.1%) at the lowest extract concentration, indicating that low doses may enhance growth rather than inhibit it. In **Figures 2B and 3**, the results of radicle length inhibition also revealed marked differences among species and concentrations. For *E. crus-galli*, the highest concentration achieved up to 93% inhibition, while lower concentrations reduced the inhibitory effect, and at the lowest concentration, the extract caused a stimulatory response in radicle growth with a value of -6.2%. *Fimbristylis miliacea* showed a similar response, with a stimulation level of -4.7% at the lowest concentration. These findings suggest that at certain concentrations, the compounds could switch from inhibitory to stimulatory effects, reflecting the dose-dependent nature of the plant extract's action. From a concentration of 0.24 g $mL^{-1}$, the *C. bipinnatus* extract started to significantly inhibit the growth of weeds, especially *L. chinensis* and *F. miliacea* (reaching $IC_{90}$), while *E. crus-galli* and *O. sativa* f.

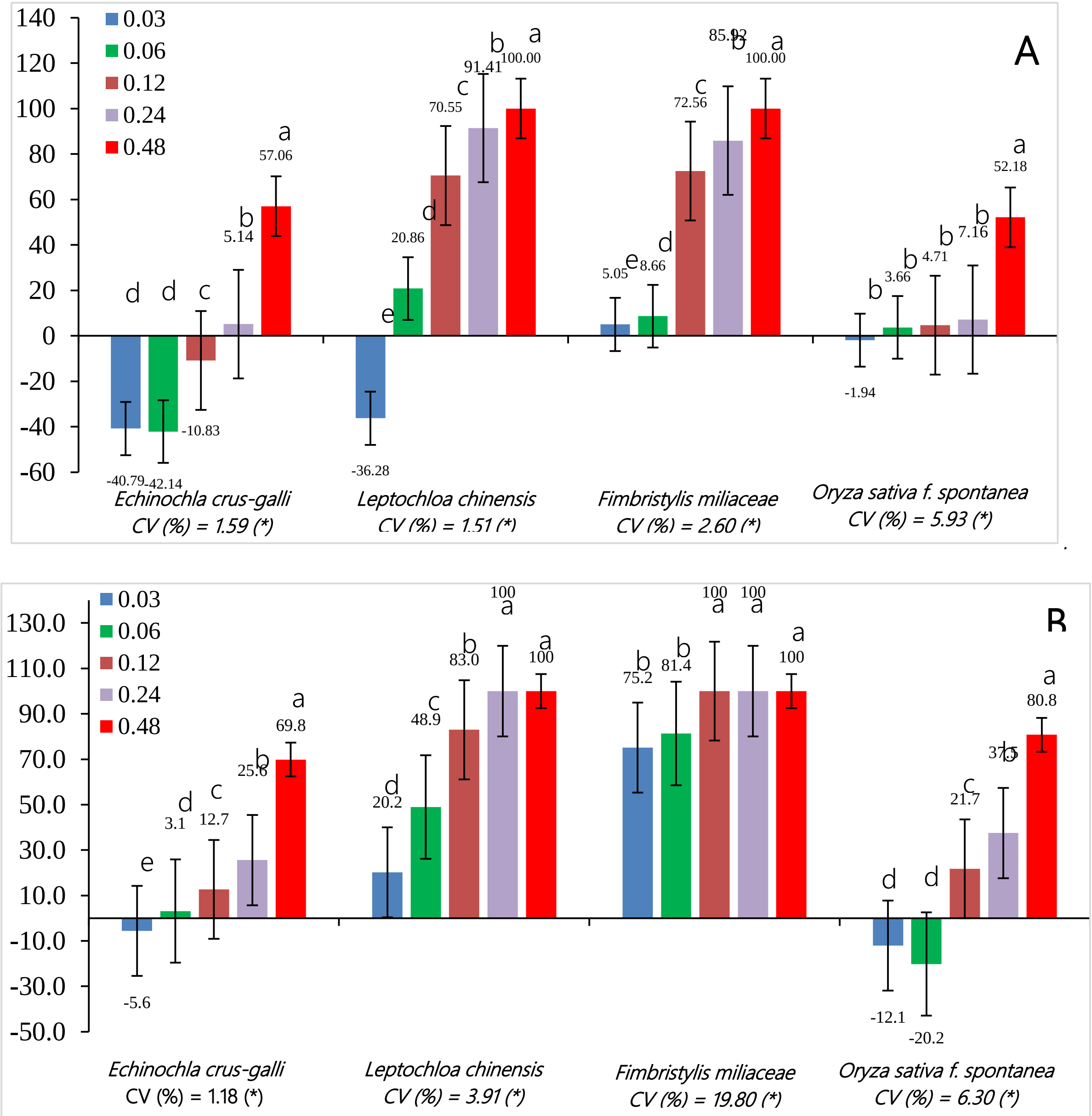



*Note: Means with the same letter within a column are not significantly different according to Duncan's multiple range test, * indicates significant difference at the 5% level. Negative values in the graph indicate stimulation.*

**Figure 1.** Inhibition percentages of the *Cosmos bipinnatus* extract at various concentrations on the hypocotyl and radicle lengths of *Brassica juncea* seedlings after 48 hours

*spontanea* were only strongly inhibited at a higher concentration of 0.48 g mL$^{-1}$.

The bioactivity of the *C. bipinnatus* extract on several widely cultivated rice cultivars in the Mekong Delta at a concentration of 0.03 g mL$^{-1}$ exhibited a slight stimulatory effect on all three rice cultivars after 48 hours. Nevertheless, at a concentration of 0.06 g mL$^{-1}$, inhibition was observed in OM380 (32.5%), while OM5451 and OM18 continued to display mild stimulation of -17.6% and -2.9%, respectively. When the concentration was elevated to 0.12 g mL$^{-1}$, all three cultivars showed signs of inhibition, with marked suppression of radicle growth at higher concentrations. Specifically, OM5451 experienced severe inhibition (98.7%) at a concentration of 0.48 g mL$^{-1}$, OM380 was inhibited at both 0.24 and 0.48 g mL$^{-1}$, and OM18 exhibited inhibition of 77.2% (**Figures 4 and 5**).




*Note: Means with the same letter in the same column are not significantly different according to Duncan's test, * significantly different at the 5% level. Negative values in the graph indicate stimulation.*

**Figure 2.** Effects of different concentrations of the Cosmos bipinnatus extract on the shoot **(A)** and root lengths **(B)** of various weed species after 48 hours of treatment

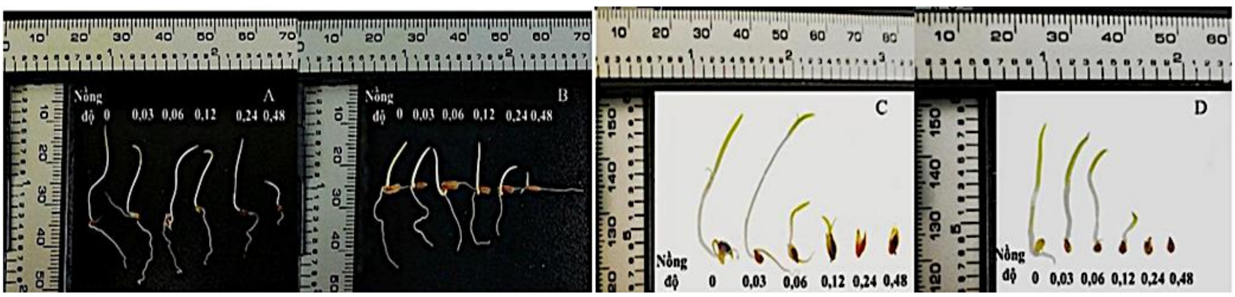



**Figure 3.** The impacts of varying concentrations of the *Cosmos bipinnatus* extract on *Echinochloa crus-galli* **(A),** *Oryza sativa* f. *spontanea* **(B),** *Leptochloa chinensis* **(C),** and *Fimbristylis miliacea* **(D)** after 48 hours of treatment

The inhibitory effects on shoots were similar to those on roots, suggesting that the OM380 rice variety was quite sensitive to the *C. bipinnatus* extract (except for the 0.03 g $mL^{-1}$ dose, which stimulated -4.55% shoot growth). Notably, the 0.24 g $mL^{-1}$ dose caused 74.64% inhibition, which increased to 95.44% at the 0.48 g $mL^{-1}$ dose. Similarly, in the OM5451 rice variety, the 0.48 g $mL^{-1}$ dose caused 84.02% inhibition, while lower doses (0.03-0.24 g $mL^{-1}$) stimulated growth inconsistently with increasing dose. For OM18, both the 0.03 and 0.06 g $mL^{-1}$ doses stimulated -6.95% shoot growth, indicating that this variety had a higher tolerance and effective degradation of the inhibitory effects of the *C. bipinnatus* extract (**Figures 4 and 5**).

**Correlation between the *Cosmos bipinnatus* extract dosages and their allelopathic potentials**

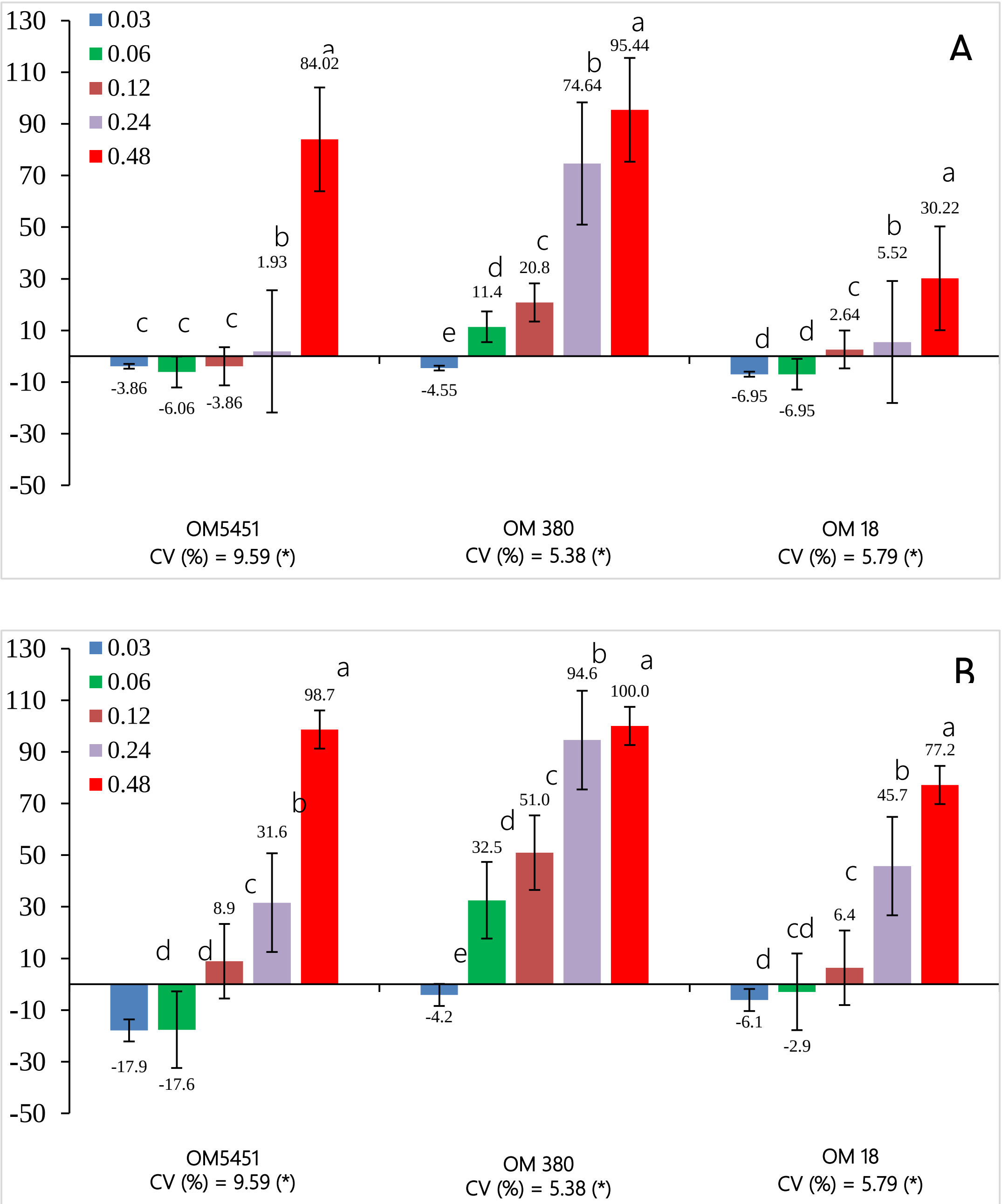
The results from **Figure 6** assess the impacts of the *C. bipinnatus* extract doses on the inhibition of radicle and coleoptile growth in the common weeds *E. crus-galli*, *L. chinensis*, *F. miliaceace*, and *O. sativa* f. *spontanea*. The graphical results indicated a positive correlation between extract dosage and the inhibition rate of both the radicles and coleoptiles of the weeds, with the coefficients of determination ($R^2$) and *P*-values demonstrating statistical significance. The x-axis represents the extract dosage (X) in g $mL^{-1}$, while the y-axis represents the inhibition rate (Y) of weed growth. An $R^2$ value close to 1 indicates that the regression model explains the data variability well, while a *P*-value <0.01 confirms a high level of statistical significance.

For *E. crus-galli*, the results showed a strong inhibitory effect of the extract with $R^2$ = 0.9908 on the roots and 0.9747 on the shoots with *P* <0.01, demonstrating that as the dosage increased, the inhibition rate also increased significantly. This indicated the potential of *C. bipinnatus* in controlling this weed species. Similarly, *L. chinensis* also showed a significant response to the extract with $R^2$ = 0.611 on the roots and 0.618 on the shoots with *P* <0.05, although the efficacy was lower compared to *E. crus-galli*. The reason may be due to the differences in the physiological structure and tolerance of each species. For *O. sativa* f. *spontanea* (weedy rice), $R^2$ = 0.9314 on the roots and 0.8923 on the shoots with *P* <0.05, indicating a high control efficacy, nearly equivalent to *E. crus-galli*. Meanwhile, *F. miliaceace* showed the lowest efficacy among the studied species with $R^2$ = 0.4932 on the roots and 0.7055 on shoots with *P* <0.01, which may be related to the natural resistance of *F. miliaceace* to allelopathic compounds.

**Figure 7** illustrates the correlation between the *C. bipinnatus* extract dosages and the inhibition rates of radicle and coleoptile growth in the OM rice varieties. The linear regression lines show a positive correlation between extract dosage and inhibition rate, indicating that higher concentrations of the extract led to stronger inhibition of growth. The $R^2$ coefficients quantified the strength of this relationship, demonstrating a high correlation between dosage and inhibition rate for both radicles and coleoptiles. Statistically significant *P*-values supported the reliability of these correlations, confirming that the extract dosage was a critical factor influencing growth inhibition in the OM rice varieties. This suggests that the *C. bipinnatus* extract dosage had a measurable and significant impact on the suppression of root and shoot growth, providing insights into optimal concentrations for selective weed control.

**Quantification of total phenolic and flavonoid contents in different parts of *Cosmos bipinnatus***

The total phenolic and flavonoid contents in different parts of C. bipinnatus varied significantly (**Table 1**). The whole plant (SNC Total Sample) exhibited the highest levels of phenolics and flavonoids, with 94.48 mg GAE $g^{-1}$ and 514.61 mg QE $g^{-1}$, respectively. Among the individual plant parts, the flowers contained the second-highest levels, with 63.89 mg GAE $g^{-1}$ of phenolics and 127.74 mg QE $g^{-1}$ of flavonoids. Leaves followed, containing 45.93 mg GAE $g^{-1}$ of phenolics and 107.13 mg QE $g^{-1}$ of flavonoids. The roots and stems had the lowest phenolic and flavonoid levels, with roots showing 26.08 mg GAE $g^{-1}$ of phenolics and 69.76 mg QE $g^{-1}$ of flavonoids, and stems containing 19.21 mg GAE




*Note: Means with the same letter in the same column are not significantly different according to Duncan's test, * significantly different at the 5% level. Negative values in the graph indicate stimulation.*

**Figure 4.** Inhibition percentages of the Cosmos bipinnatus extract at different concentrations on the shoot (A) and root lengths (B) of OM rice varieties after 48 hours of treatment

$g^{-1}$ of phenolics and 41.07 mg QE $g^{-1}$ of flavonoids. These differences were statistically significant at the 5% level according to Tukey's test.

## Dicussion

The differential susceptibility of the weed species followed the order: *Fimbristylis miliacea* < *Leptochloa chinensis* < *Echinochloa crus-galli* < *Oryza sativa f. spontanea*, suggesting species-specific concentrations of *Cosmos bipinnatus* extract may optimize weed control (Chon *et al.*, 2003; Weston & Duke, 2003). These results support findings on phenolic and flavonoid compounds having dose-dependent effects either inhibitory or stimulatory (Gebreyohannes *et al.*,

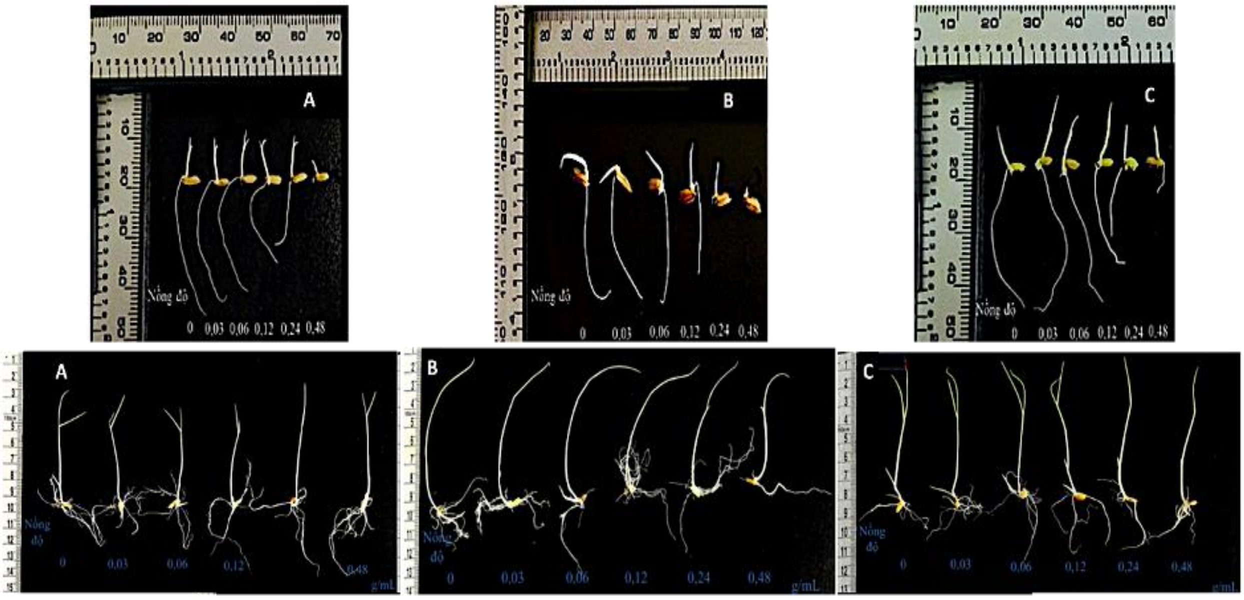
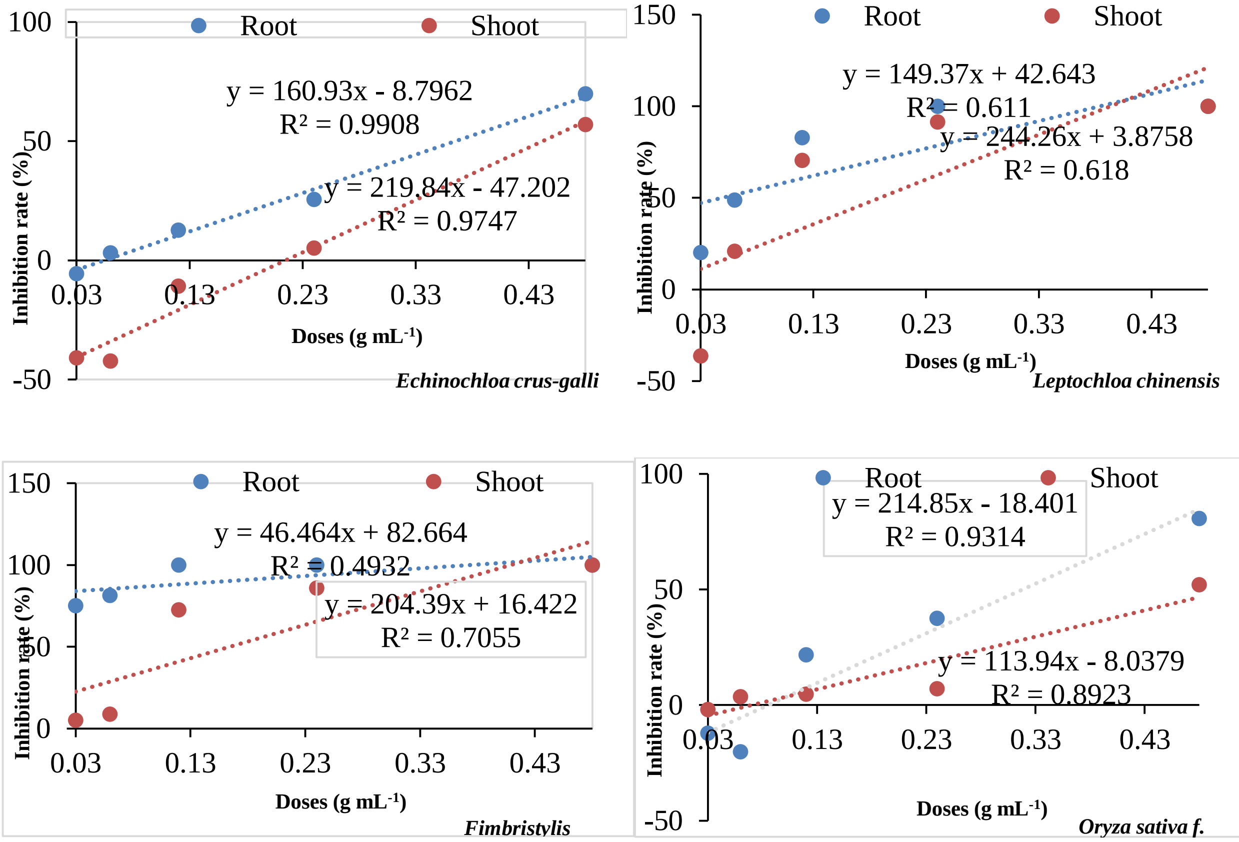
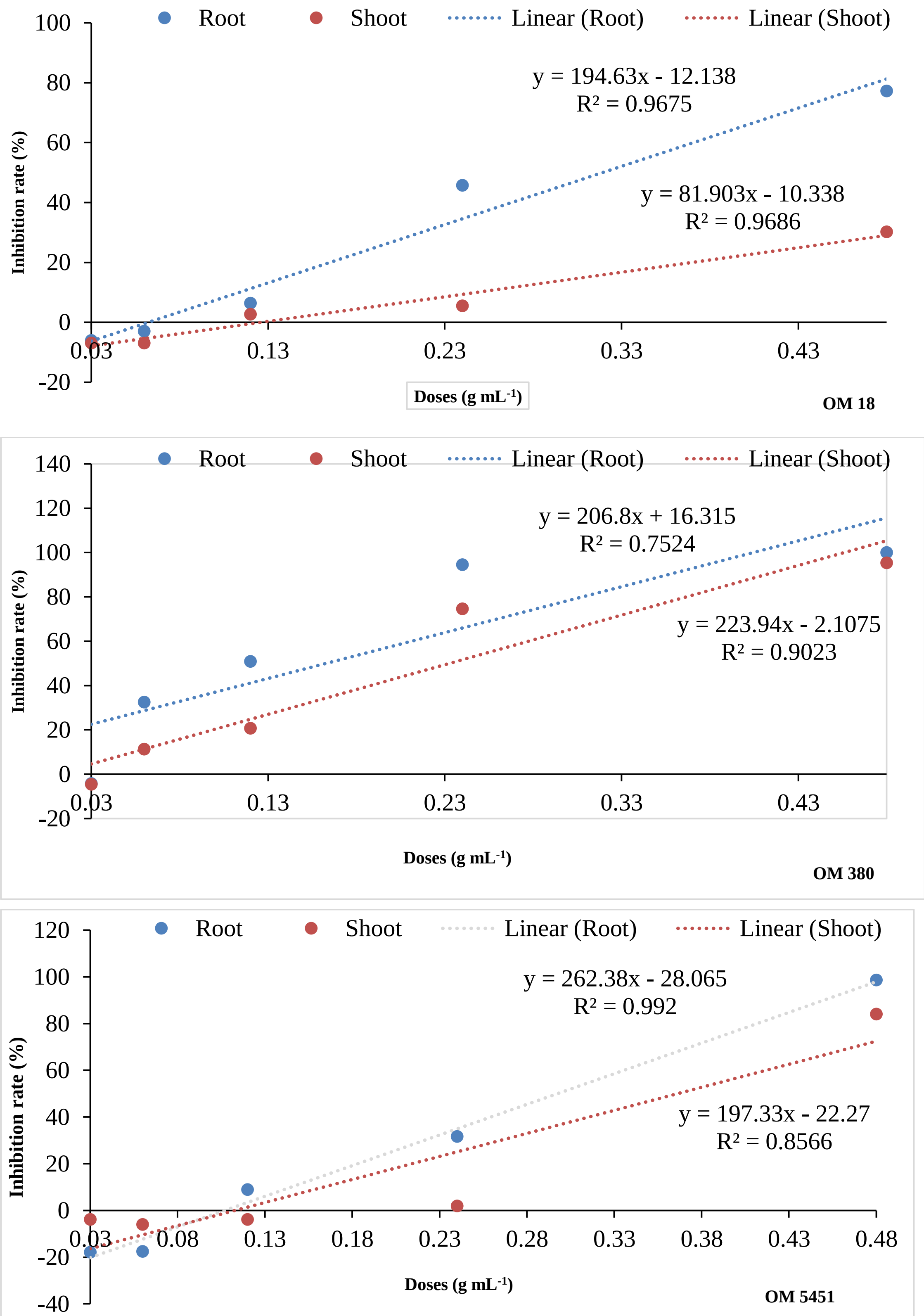



**Figure 5.** Effects of the *Cosmos bipinnatus* extract on rice cultivars OM5451 (A), OM380 (B), and OM18 (C) at 48 hours (upper figures) and recovery ability at 168 hours (lower figures) after treatment at concentrations of 0.00 g $mL^{-1}$ (control), 0.03 g $mL^{-1}$, 0.06 g $mL^{-1}$, 0.12 g $mL^{-1}$, 0.24 g $mL^{-1}$, and 0.48 g $mL^{-1}$




*Note: Linear regression analysis demonstrates the effect of extract concentration on the inhibition rate. The $R^2$ values indicate the correlation between the independent variable x (extract concentration) and the dependent variable y (inhibition rate of radicle and coleoptile), while the P-values represent the statistical significance of each relationship.*

**Figure 6.** Correlations between the Cosmos bipinnatus extract concentrations and the inhibition of radicle and coleoptile growth in weeds




*Note: The linear regression line indicates the level of influence of the extract dosage on the inhibition rate. The $R^2$ coefficients show the correlation level between the independent variable x (extract dosage) and the dependent variable Y (inhibition rate of radicle and coleoptile), where the P-values represent the statistical significance of each relationship.*

**Figure 7.** Correlations between the Cosmos bipinnatus extract dosages and the inhibition of radicle and coleoptile growth in the OM rice varieties

**Table 1.** Total phenolic and flavonoid contents in different parts of *Cosmos bipinnatus*

| Extract type | Total phenolics (mg GAE $g^{-1}$ extract) [(1)] | Total flavonoids (mg QE $g^{-1}$ extract) [(2)] |
|---|---|---|
| SNC total sample | $94.48^{a} \pm 6.09$ | $514.61^{a} \pm 2.19$ |
| SNC roots | $26.08^{d} \pm 3.43$ | $69.76^{d} \pm 1.05$ |
| SNC stems | $19.21^{d} \pm 2.60$ | $41.07^{e} \pm 2.13$ |
| SNC leaves | $45.93^{c} \pm 4.05$ | $107.13^{c} \pm 4.26$ |
| SNC flowers | $63.89^{b} \pm 5.67$ | $127.74^{b} \pm 3.05$ |

*Note: (1) Values in this column were determined based on the standard curve equation of gallic acid (y = 0.0657x + 0.0075; $R^2$ = 0.9978); (2) values in this column were determined based on the standard curve equation of quercetin (y = 0.0055x – 0.0117; $R^2$ = 0.9978). Within the same column, mean values followed by the same letter do not differ significantly at the 5% level according to Tukey's test.*

2023). The slight stimulation observed at low concentrations confirms the allelopathic potential of *C. bipinnatus* and highlights the importance of dosage in sustainable weed management (Fujii *et al.*, 2004; Madhavi *et al.*, 2017; Li *et al.*, 2021).

In rice systems, *C. bipinnatus* extracts exhibited dual effects growth inhibition at high concentrations and stimulation at low concentrations indicating its potential application in weed suppression and rice productivity enhancement. At higher doses, coleoptile and radicle growth of OM5451 and OM380 were notably suppressed, aligning with prior allelopathic findings that demonstrate how natural plant compounds chemically inhibit others (Xuan *et al.*, 2005; Kong *et al.*, 2007). Thus, high-concentration applications pose phytotoxic risks to rice and require precise control.

Conversely, at lower extract concentrations, positive growth effects were observed in rice cultivars, suggesting potential as a bio-stimulant. Low-dose stimulation is supported by other allelopathic research (Huang *et al.*, 2013). These results emphasize the importance of application strategy, as even minor deviations in dosage could shift the effect from beneficial to detrimental (Cheng & Cheng, 2015). Recovery patterns of rice cultivars (OM5451, OM380, OM18) after 168 hours of treatment (**Figures 5A–C**) revealed substantial resilience. Although early growth (48 hours) was suppressed, rice recovered significantly by 168 hours, likely due to internal regulation mechanisms that allow adaptation after allelopathic stress (Xuan *et al.*, 2006; Cheng & Cheng, 2015). This resilience indicates that *C. bipinnatus* extracts, if appropriately dosed, could be utilized in rice fields without long-term damage to crops.

This finding also suggests a selective window of application, where rice shows temporary suppression but rebounds later, minimizing yield loss. This selective inhibition, combined with crop recovery, supports integrating *C. bipinnatus* into biological weed control programs, especially where chemical herbicides are problematic (Kong *et al.*, 2007). Further exploration into rice's recovery mechanisms could contribute to developing resistant varieties or timing guidelines for extract application (Huang *et al.*, 2013). **Figure 6** further supports the potential of *C. bipinnatus* as a natural herbicide, promoting the shift from synthetic to environmentally friendly weed control strategies (Anjum *et al.*, 2010). The study by Hussain & Gadoon (2011) noted similar weed-suppressing activity from Asteraceae flavonoids, reinforcing our findings. Anjum *et al.* (2010) reported *C. bipinnatus* extracts could inhibit *Triticum aestivum*, further evidencing the species' bioactivity.

Low concentrations of the extract also showed hormesis-like responses in rice mild stimulation followed by inhibition at higher doses. This dose-dependent behavior is typical of hormesis, a well-documented phenomenon in plant allelopathy (Anaya, 1999; Duke *et al.*, 2006). It opens the possibility of using the extract as both a bio-stimulant and herbicide, depending on concentration. In early stages, low-dose applications could enhance rice growth while reducing weed competition (Khanh *et al.*, 2005; Scavo & Mauromicale, 2021).

The extract's high phenolic and flavonoid contents, particularly in leaves and flowers, support its strong allelopathic effects. The whole plant exhibited the highest concentrations, with 94.48 mg GAE/g phenolics and 514.61 mg QE/g flavonoids (**Table 1**), consistent with the correlation between such compounds and allelopathic potential (Madhavi *et al.*, 2017). Phenolic compounds disrupt weed development via enzyme inhibition and photosynthetic interference (Inderjit & Dakshini, 1991; Sahu *et al.*, 2019).

The distribution of these compounds across plant parts mirrors other Asteraceae members. Leaves (44.34 mg GAE $g^{-1}$ and 113.80 mg QE $g^{-1}$) and flowers (63.57 mg GAE $g^{-1}$ and 135.62 mg QE $g^{-1}$) showed significantly higher concentrations than roots. Asha & Sivaji (2015) and Xiong *et al.* (2020) similarly found that flower and leaf extracts exert stronger allelopathic effects. Roots, lower in secondary metabolites, are likely less bioactive due to their primary function in water/nutrient absorption. Consequently, aerial parts should be prioritized for extract-based product development. Supporting this, Ghassemi-Golezani *et al.* (2019) found that leaf and flower extracts of other species reduced weed growth by up to 70%, underlining *C. bipinnatus*'s potential as a raw material for bioherbicide development. These findings justify the exploration of leaf and flower-derived formulations for integrated weed management in organic rice farming.

In summary, C. bipinnatus exhibits concentration-dependent allelopathic activity, with potential as a natural herbicide and bio-stimulant. Its application requires careful control to maximize weed suppression while minimizing crop stress. The plant's high phenolic and flavonoid content, especially in above-ground parts, highlights its value for sustainable agriculture. Future research should assess long-term field performance, interactions with soil microbiota, and formulation development to translate these findings into practical applications.

## Conclusions

This study demonstrated that *C. bipinnatus* is a promising biological resource for weed control due to its high contents of phenolics and flavonoids, particularly in leaves and flowers. Extracts from this plant exhibited inhibitory effects on the growth of common rice field weeds, with the inhibitory effect increasing with concentration increases. However, at low concentrations, the extract could stimulate the growth of some rice varieties, suggesting its versatile application potential. To maximize the potential of *C. bipinnatus*, further studies are needed to determine the optimal dosage and timing for field applications, thereby contributing to the development of sustainable and environmentally friendly weed management strategies.

## Acknowledgments

The authors would like to express their sincere gratitude to the Ministry of Education and Training of Vietnam for providing financial support for the project code: B2024-TCT-10 to conduct this study partly.

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