Determining Allelopathic Potential of Anethum Graveolens to Conrol Chenopodium Album

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Weeds pose significant challenges in agriculture by reducing crop yields and competing for resources. Traditional herbicide use is often economically inefficient, environmentally harmful, and potentially carcinogenic. This study investigates the allelopathic potential of Anethum graveolens L. (dill) as a natural alternative for controlling Chenopodium album L. (Bathua), a fast-growing weed from the Chenopodiaceae family. Various concentrations of aqueous and methanolic extracts from A. graveolens seeds and shoots (control, 0.4%, 0.8%, 1.2%, 1.6%, and 2.0%) were tested for their effects on C. album seed germination using a Petri plate assay. The 1.5% aqueous seed extract showed the highest inhibitory effect, reducing germination by 99%, surpassing both the aqueous shoot extract (64%) and methanolic extracts. In foliar spray bioassays, aqueous and methanolic extracts (control, 4%, 8%, 12%, 16%, and 20%) were applied to one- and two-week-old C. album seedlings at seven-day intervals. A 16% concentration reduced fresh plant weight by 51% after the first week and 75% after the second week, indicating progressive phytotoxicity. Pot bioassays revealed that a 2% extract concentration (among control, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) significantly decreased shoot weight (−83%), root weight (−74%), and shoot length (−65%). These results highlight the promising allelopathic potential of A. graveolens as a bioherbicide, offering a sustainable, eco-friendly, and cost-effective alternative for weed management in agricultural systems.

Keywords: Allelopathic, Anethum graveolens, Chenopodium album. bioherbicide, weed control, aqueous extract, methanolic extract, seed germination, foliar spray, sustainable agriculture.

Introduction:

The growing need for sustainable weed control methods has led researchers to explore plant-derived allelochemicals as a natural alternative to synthetic herbicides [1]. Allelopathy, a fascinating ecological process where plants release biochemical, affect the growth of nearby species offers a promising way to manage weeds naturally [2]. These allelochemicals are found in leaves, flowers and roots of plants that enter the environment through decomposition, chemical signals in root secretions, or rainwater leaching, modifying soil conditions and may affect the plant-plant or plant-soil biology [3]. Anethum graveolens (dill), a well-known herb from the Apiaceae family, it is valued for both its culinary and medicinal uses [4]. Different parts of dill plants showed strong allelopathic properties, with its extracts showing significant growth-suppressing effects on various weeds [5]. The phytochemical analysis of the dill plant revealed the presence of many saponins, flavonoid, terpenoids, alkaloids, phenolic and tannins [6]. However, the specific impact of dill leaf extracts on C. album remains understudied. The use of plant extracts for weed control is an eco-friendly alternative to synthetic herbicides, offering reduced environmental toxicity and lower risk of herbicide resistance. Several plant species produce allelopathic compounds that inhibit weed germination and growth. Weeds present a significant challenge in agriculture by competing for vital resources such as nutrients, sunlight and water, which can severely reduce crop yields [7].

One particularly troublesome weed, Chenopodium album L. (Bathua), is a fast-spreading annual weed belonging to the Chenopodiaceae family. It is one of the most problematic weeds all over the world. It is an annual herbaceous weed found in Pakistan, India as well as other subtropical and tropical parts of Asia. It thrives in various climates, including tropical and temperate regions of Pakistan [8]. Due to rapid growth, prolific seed production and adaptability, bathua is a persistent problem for farmers, demanding sustainable and innovative control strategies [9].

This study explores the effects of methanol-extracted dill leaves on C. album, focusing on seed germination, root growth, and early seedling development. By identifying the active phytochemicals responsible for these effects, this research aims to contribute to the development of eco-friendly herbicides, providing farmers with a sustainable tool to combat C. album infestations.

Objectives:

1. To evaluate the allelopathic potential of Anethum graveolens in controlling Chenopodium album.
2. To identify the allelopathic phytochemicals present in Anethum graveolens.

Novelty Statement:

While allelopathy has been extensively studied in various plant species, this research represents one of the first systematic investigations of Anethum graveolens as a natural bioherbicide against Chenopodium album. The use of dill extracts or residues presents a sustainable weed management strategy, offering benefits such as biodegradability, cost-effectiveness, and compatibility with organic farming and integrated pest management (IPM) systems. This approach could significantly reduce dependence on synthetic herbicides and promote agroecological practices.

Materials and Methods:

Collection of Plant Material:

A multi-phase experimental approach was employed to evaluate the allelopathic potential of Anethum graveolens against Chenopodium album. Whole dill plants were collected from agricultural fields in Narowal, Pakistan (32.1°N, 74.5°E) during their peak growth season using completely randomized sampling. In the laboratory, leaves and seeds were separated, shade-dried at 25±2°C for seven days, and then ground into a fine powder.

Plant Extraction:

Two extraction methods were employed: aqueous extraction using distilled water (1:10 w/v ratio, soaked for 7 days with daily shaking) and methanolic extraction using 80% methanol (continuous shaking for 24 hours). The extracts were filtered through muslin cloth and Whatman No.1 filter paper, concentrated using a rotary evaporator at 40°C and working concentrations were prepared through serial dilution.

In Vitro Bioassay:

Three complementary bioassays were conducted to assess the allelopathic effects. In the laboratory germination test, surface-sterilized C. album seeds were placed in Petri dishes with filter paper moistened with different concentrations of the extracts (ranging from 0.4% to 2.0%). Germination was monitored daily for 15 days.

Pot Experiment:

Following in vitro assays, a pot experiment was conducted to assess the allelopathic impact of dried dill biomass on Chenopodium album growth. Sterilized soil (pH 7.2, 1.2% organic matter) was placed in 15 cm pots. Dill biomass was incorporated at concentrations ranging from 0.5% to 3% (w/w) into 23 pots, each with three replicates, prior to sowing.

Growth parameters such as shoot weight, root weight, and shoot length were recorded after 45 days.

Foliar Bioassay:

After evaluating the soil-mediated effects of dill biomass, a foliar spray assay was conducted to determine the phytotoxic potential of dill aqueous extracts when applied directly to the foliage of Chenopodium album. This assay helped compare the effectiveness of direct vs. indirect modes of allelopathic action. C. album seedlings were grown for two weeks before applying extract sprays weekly (concentration range: 4% to 20%). Fresh biomass was recorded after the first and second weeks of spraying.

Statistical Analysis:

The experiments followed a completely randomized design (CRD), with three replicates per treatment including controls. Data were analyzed using ANOVA, and treatment differences were determined through LSD post-hoc tests ($p<0.05$) using SPSS version 26. This statistical approach enhanced the validity and reliability of the results by providing a clearer understanding of specific group differences.

Flow Diagram:

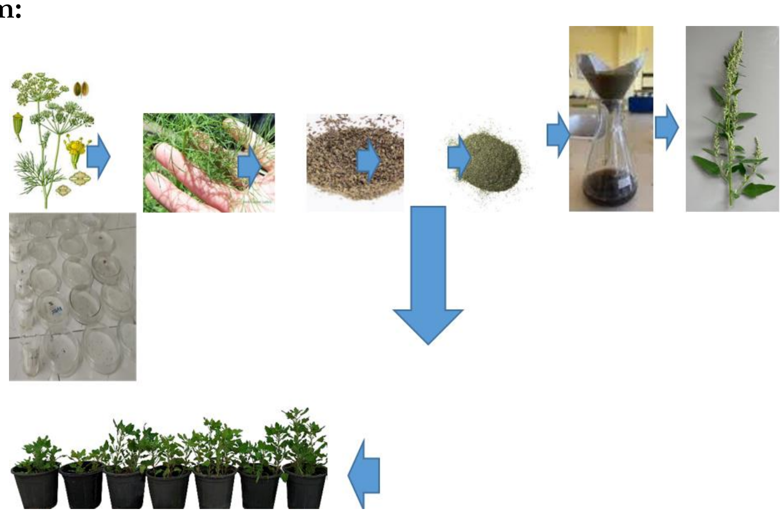

Figure 1. Flow diagram of methodology

Results:

In vitro Germination Assay:

The allelopathic stress of methanolic (M) and aquous (W) extract of Anethum graveolens seeds:

The seed germination results clearly demonstrated the strong inhibitory effects of Anethum graveolens extracts on Chenopodium album (Figure 2). Both aqueous (W) and methanolic (M) extracts significantly reduced germination rates ($p < 0.05$), with aqueous extracts exhibiting particularly potent effects. The 1.5% aqueous concentration led to a remarkable 99.8% inhibition of C. album seed germination, while even the lowest tested concentration (0.4%) resulted in a 14% reduction. Although methanolic extracts also showed inhibitory activity, their effects were generally weaker across all concentrations compared to the aqueous extracts. Statistical analysis confirmed that each treatment produced significantly distinct effects, with aqueous extracts consistently outperforming their methanolic counterparts at equivalent concentrations.

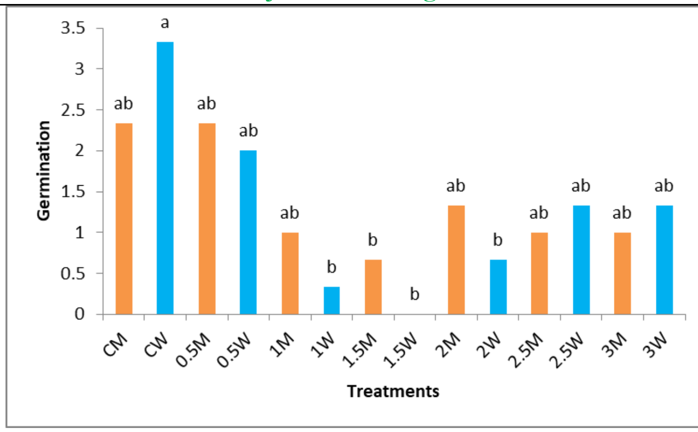

Figure 2. Effect of Anethum graveolens seeds aqueous and methanolic extracts on seed germination of Chenopodium album. Values with different letters at their top show significant difference (P≤O.O5) as determined by LSD Test

The allelopathic stress of aqueous and methanolic extracts Anethum graveolens shoot:

Analysis of shoot extracts revealed more pronounced effects compared to seed extracts (Figure 3). Both aqueous and methanolic shoot extracts of Anethum graveolens significantly inhibited Chenopodium album seed germination ($p < 0.05$). However, the overall effects were weaker than those observed with leaf extracts. The most effective treatment was the 0.5% aqueous shoot extract, which reduced seed germination by approximately 64%. Across all tested concentrations, aqueous shoot extracts consistently inhibited germination by 21–64%, whereas methanolic extracts showed more variable and less consistent results. Each treatment produced distinct effects, with significant variation depending on the concentration and extraction method.

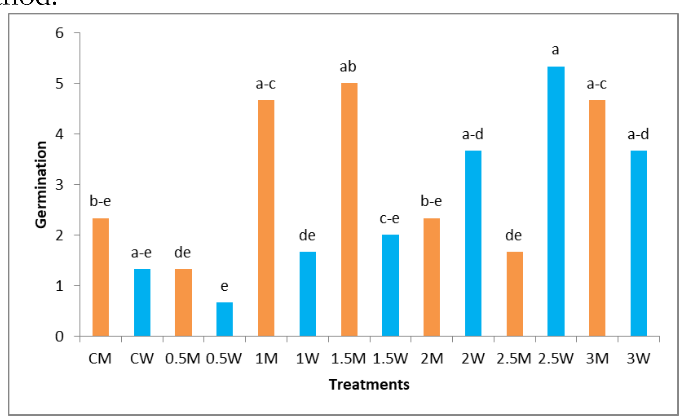

Figure 3. Effect of Anethum graveolens shoot aqueous and methanolic extracts on seed germination of Chenopodium album. Values with different letters at their top show significant difference (P≤O.O5) as determined by LSD Test

Spray Bioassay:

Plant Fresh Biomass in First Week Spray:

The foliar spray treatment showed significant effects on Chenopodium album biomass (Figure 4). After just one week of application, a statistically significant reduction in fresh plant weight was observed ($p < 0.05$). Untreated control plants exhibited the highest biomass (0.69 g), while treated plants showed progressively lower weights with increasing extract concentrations. Biomass decreased from 0.64 g (a 7% reduction) at 4% concentration to 0.34 g (a 51% reduction) at the highest concentration of 16%. The effects were particularly pronounced at higher concentrations, with the 16% foliar spray causing the most substantial suppression of plant growth. Each concentration yielded statistically distinct results, demonstrating a clear and consistent concentration-dependent allelopathic effect.

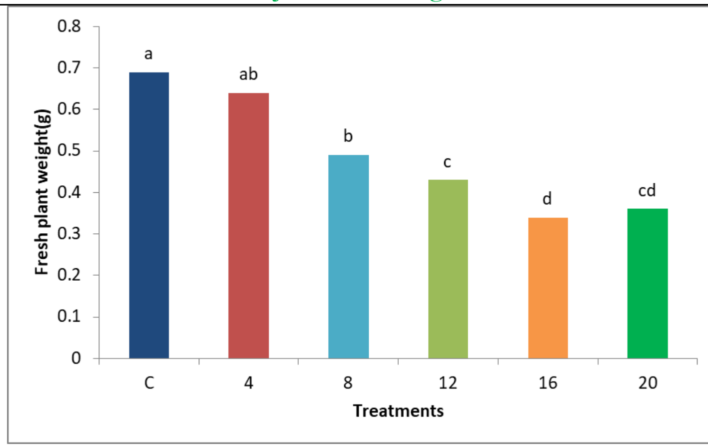

Figure. 4. Plant fresh biomass of Chenopodium album in first week of foliar spray of aqueous extracts of Anethmn graveolens. Values with different letters at their top show significant difference (P≤O.O5) as determined by LSD Test

Plant fresh biomass in second week spray:

The cumulative effects of repeated foliar applications became more apparent in the second week of the spray bioassay (Figure 5). Control plants maintained robust growth, with a fresh weight of 0.6 g. In contrast, a striking suppression of Chenopodium album biomass was observed across all treatment concentrations ($p < 0.05$). The inhibitory effects intensified with both increasing concentration and prolonged exposure—ranging from an 11% reduction (0.53 g) at the mildest 4% treatment to a remarkable 75% reduction (0.3 g) at the highest 16% concentration. This progressive decline suggests that the allelochemicals present in Anethum graveolens not only exert immediate phytotoxic effects but may also accumulate in plant tissues over time, enhancing their impact. Once again, the 16% concentration proved to be the most effective, consistently demonstrating strong phytotoxic activity across repeated applications. Statistical analysis confirmed that each concentration produced distinct and measurable effects on plant biomass.

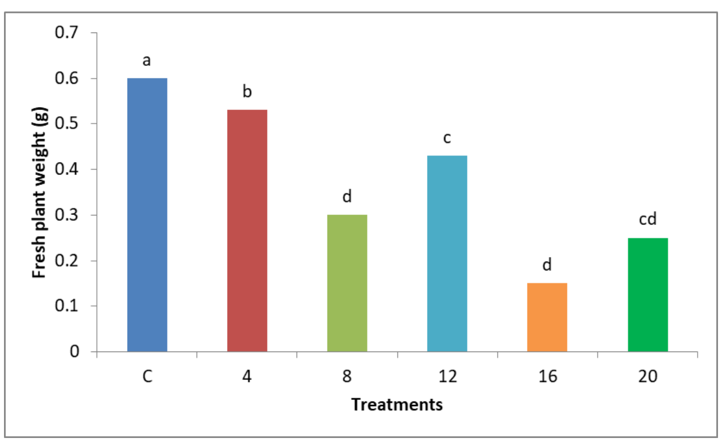

Figure 5. Plant fresh biomass of Chenopodium album in second week of foliar spray of aqueous extracts of Anethmn graveolens. Values with different letters at their top show significant difference (P≤O.O5) as determined by LSD Test

Pot Bioassay:

Effect of Anethum Graveolens Dry Biomassm on Shoot Weight of Chenopodium album:

The pot experiment revealed striking effects of Anethum graveolens extracts on Chenopodium album shoot weight (Figure 6). Control plants exhibited vigorous growth, with an average shoot weight of 1.11 g. In contrast, treated plants showed progressively suppressed growth as extract concentrations increased ($p < 0.05$). Even the lowest concentration (0.5%) reduced shoot weight by 28% (0.79 g), demonstrating the potency of the allelochemicals present in the extracts. The effects became dramatically more pronounced at higher concentrations; the 2% treatment resulted in an 83% reduction in shoot biomass (0.18 g), indicating near-complete growth inhibition. This clear dose-response relationship, where increased concentrations produced significantly different effects, strongly supports the phytotoxic potential of A. graveolens extracts against this problematic weed species.

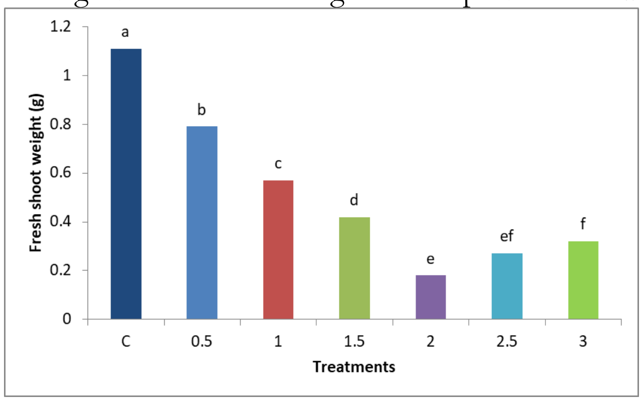

Figure 6. Effect of Anethmn graveolens on shoot weight of Chenopodium album Values with different letters at their top show significant difference (P≤O.O5) as determined by LSD Test

Effect of Anethum graveolens dry biomassm on Root weight of C album:

The root system of Chenopodium album proved particularly sensitive to Anethum graveolens treatments, as shown in Figure 7 ($p < 0.05$). While untreated control plants developed healthy roots with an average biomass of 1.02 g, a progressive decline in root biomass was observed with increasing extract concentrations. Even the mildest treatment (0.5%) caused a measurable 13% reduction (0.88 g), suggesting that A. graveolens allelochemicals rapidly impact root development.

The inhibitory effects intensified significantly with higher concentrations, culminating in a 74% reduction in root biomass (0.26 g) at the 2% treatment level. This trend indicates that A. graveolens not only suppresses above-ground growth but may more severely impair root systems, which are critical for weed survival and competitiveness. Each concentration tested produced statistically distinct effects, confirming the reliability of these observations and underscoring the potential of A. graveolens extracts as a comprehensive weed control solution targeting both shoot and root development.

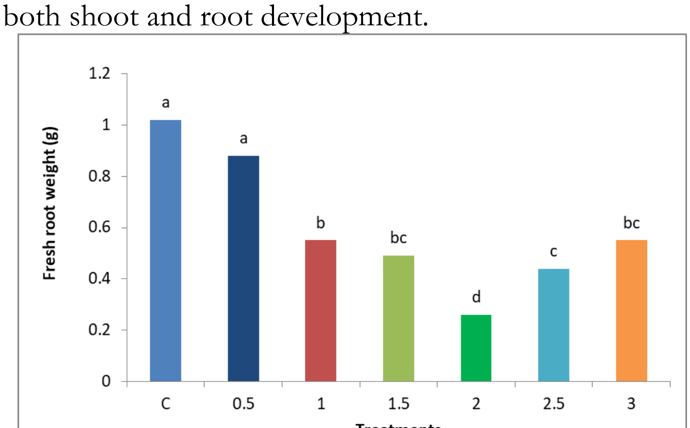

Figure 7. Effect of Anethmn graveolens on root weight of Chenopodium album Values with different letters at their top show significant difference (P≤O.O5) as determined by LSD Test

Effect of Anethum graveolens dry biomass on Shoot length of Chenopodium album:

The stunting effects of Anethum graveolens extracts on Chenopodium album became visually apparent in shoot length measurements (Figure 8). Control plants grew vigorously, reaching an average height of 37.27 cm, while treated plants exhibited progressively stunted growth with increasing extract concentrations ($p < 0.05$). This inhibitory effect followed a clear dose-response pattern—even the lowest concentration (0.5%) reduced shoot length by 16% (31.09 cm), highlighting the sensitivity of C. album to these allelochemicals.

The most dramatic effects were observed at the 2% concentration, where plants reached only 12.73 cm—65% shorter than the controls—and exhibited severely stunted growth with noticeably shorter internodes. What is particularly interesting is that this growth suppression occurred consistently across all tested concentrations, with each treatment level producing statistically distinct results. These findings suggest that A. graveolens extracts not only reduce biomass but also actively interfere with fundamental growth processes in C. album, potentially by disrupting cell elongation or hormonal regulation.

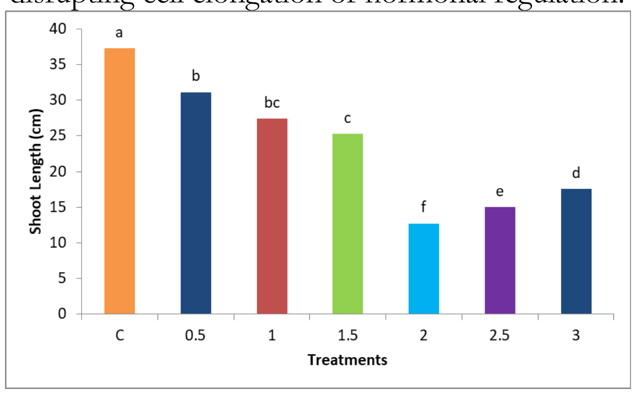

Figure 8. Effect of Anethmn graveolens on shoot length of Chenopodium album Values with different letters at their top show significant difference (P≤O.O5) as determined by LSD Test

Tabular form:

Discussion:

The present study demonstrates the strong allelopathic potential of Anethum graveolens extracts to suppress the germination and growth of Chenopodium album, supporting the hypothesis that plant-derived allelochemicals can serve as effective bioherbicides. Notably, the aqueous seed extract of A. graveolens at 1.5% concentration exhibited particularly potent activity, inhibiting C. album germination by 99%. This aligns with earlier studies reporting that seed extracts contain bioactive compounds such as phenolics, terpenoids, and flavonoids [10], which act as defense agents against various biotic and abiotic stresses [11], disrupting cellular processes in target plants [12].

The marked inhibition of seed germination suggests that A. graveolens seed extracts may interfere with essential metabolic pathways required for seed imbibition and radicle emergence. Similar observations have been made by [1] and [13], who reported that allelochemicals can impair enzymatic activity and alter membrane permeability during seed germination. The comparatively lower efficacy of methanolic extracts may be attributed to differences in compound solubility. Water-soluble allelochemicals, such as phenolic acids, are more easily absorbed by seeds during early germination, while methanol extracts may include compounds that are less bioavailable at this stage [14].

Foliar spray bioassays revealed that higher concentrations (e.g., 16%) of A. graveolens extracts significantly reduced the fresh biomass of C. album (by 51–75%), indicating post-emergence herbicidal activity. These effects may result from phytotoxic compounds penetrating the leaf surface and disrupting key physiological processes such as photosynthesis, as reported in other allelopathic studies [15]. The progressive decline in plant biomass over the two-week spray period suggests cumulative damage to cellular structures and interference with vital metabolic pathways, including chlorophyll synthesis and nutrient uptake [3,16].

In pot experiments, a 2% concentration of A. graveolens extract resulted in drastic reductions in C. album shoot weight (−83%), root weight (−74%), and shoot length (−65%), confirming systemic growth inhibition. Root suppression is especially critical, as allelochemicals in the soil can inhibit root cell division and elongation, limiting water and nutrient absorption [17]. These effects are likely attributable to specific phenolic acids (e.g., ferulic and coumaric acids) or essential oil components (e.g., carvone and limonene), which have been previously shown to disrupt mitochondrial function and induce oxidative stress in weeds [18].

Overall, these findings support the potential of A. graveolens as a natural herbicide for controlling C. album, thereby reducing dependency on synthetic herbicides, which pose environmental and health risks. Future research should aim to isolate and characterize the specific active compounds in A. graveolens, and evaluate their efficacy under field conditions to assess practical applications. Additionally, exploring synergistic effects with other allelopathic plants may enhance the effectiveness of plant-based weed suppression strategies [1].

Conclusion:

The present study clearly demonstrates that Anethum graveolens possesses significant allelopathic potential against Chenopodium album, making it a promising candidate for sustainable weed management. The 1.5% aqueous seed extract was especially effective, achieving nearly complete (99%) inhibition of germination. In addition, foliar spray treatments (16%) and soil applications (2%) caused substantial reductions in seedling growth, biomass, and shoot/root development. These findings suggest that A. graveolens produces potent bioactive compounds—likely phenolics, terpenoids, or other secondary metabolites—that interfere with critical physiological processes in C. album, including germination, root development, and photosynthetic efficiency. Continued research into its allelopathic mechanisms and field-scale application could pave the way for novel, eco-friendly weed control solutions.

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