

ALLELOPATHIC INTERACTIONS IN PLANT INVASIONS: THE IMPACT OF  
*HIRSCHFELDIA INCANA* ON *HELIANTHUS ANNUUS* GERMINATION

By

Kyrollos Salama

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APPROVED

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Dr. Kate Ostevik  
Department of Evolution Ecology & Organismal Biology

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Dr. Richard Cardullo, Howard H Hays Jr. Chair and Faculty Director, University Honors Interim  
Vice Provost, Undergraduate Education

## ABSTARCT

In this research, we are studying the allelopathic effects between the common Sunflower (*Helianthus annuus*) and Shortpod Mustard (*Hirschfeldia incana*). Allelopathy is the synthesis of chemicals by a plant species that may impact nearby plants or soil microbes. It is used to suppress weeds from growing, in insect pest management, and to control diseases in field crops. However, allelopathy also allows many invasive plants to invade native habitats. Black Mustard (*Hirschfeldia incana*) is one of the common invasive species in California. Several studies have shown that planting sunflower seeds after Brassica reduces the sunflowers chances of germination and growth. Although sunflowers and Brassica share allelopathic traits, this research will be testing if *Hirschfeldia incana* affects *Helianthus annuus* seed germination and growth. In order to test this, We collected Brassica leaves from the field and prepared a leachate using the Brassica leaves. Then, we conducted germination trials using previously collected *Helianthus annuus* seeds with and without Brassica leachate. Overall, the research goal was to learn more about the allelopathic effect on these plants and how they affected their ecosystems.

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## INTRODUCTION

Allelopathy, the production of chemicals by a plant species that might influence nearby plants or soil microbes, is an important functional characteristic that can change neighbor plant performance and eventually plant structure and function. The allelopathy phenomenon was identified for the first time in the late 1930s by Hans Molisch as the influence of one plant on another through the release of chemicals into the environment (Molisch 1937). It was further characterized as any direct or indirect harmful or helpful influence of one plant (including microbes) on another through the synthesis of chemical substances that are released into the environment (Rice 1984).

Allelopathy significantly influences the spread of invasive plants, acting as a key factor for species like *Hirschfeldia incana* to dominate new territories. These invasive plants release chemicals that inhibit the growth of surrounding native flora, thereby gaining an upper hand in these environments. This chemical interaction not only affects individual species but also extends its impact to whole ecosystems. Invasive species can change the composition of native plant communities, disrupting local food chains and nutrient cycles. Moreover, allelopathic activities can lead to drastic changes in soil microbial populations, affecting soil quality and nutrient dynamics. These alterations can create a self-reinforcing cycle, further solidifying the presence of invasive species and complicating restoration efforts. A thorough understanding of allelopathic relationships in plant invasions is crucial for effective ecological conservation and for anticipating the long-term effects of invasive species on biodiversity and ecosystem functions.

The application of allelopathy in agriculture is emerging as a sustainable alternative to traditional weed control methods. Utilizing allelopathic plants or their by-products can naturally curb weed growth, diminishing the dependence on chemical herbicides. This method aligns with eco-friendly farming practices and aids in maintaining ecological balance and soil integrity. For example, incorporating allelopathic cover crops into crop rotations can manage weeds effectively while improving soil fertility. Identifying specific allelochemicals and understanding how they work could lead to new, environmentally safe herbicides. However, leveraging allelopathy in agricultural settings requires careful evaluation of its effects on non-target species and the overall environmental impact. Research in this domain is poised to offer key insights into the best combinations and sequences of crops for efficient weed control, contributing to more sustainable and ecologically conscious farming methods.

Invasive species adopt a wide array of strategies to establish in new habitats. Among these qualities is the capacity to create allelopathic compounds that can directly restrict neighboring native plants or indirectly depress native plants via disruption of beneficial belowground microbial mutualisms or changed soil resources. Allelopathy is most likely to be associated with non-native plant invasion, which means that most invasive species spread faster because of their allelopathy. Allelopathy has become well-known in the field of invasion biology as one of the possible weapon traits in the novel weapon hypothesis because of these potential negative impacts on neighbor plant fitness (Callaway and Aschehoug 2000; Callaway and Ridenour 2004; Inderjit et al. 2008). The physiology and rate of population development of native species are known to be altered by non-native invaders, as are the abundance of species within a community and even the stable states of entire ecosystems (Wardle et al. 1998, Bialic-Murphy et al. 2019,

Roche et al. 2020, and Zhang et al. 2019). Although there are obvious negative effects on specific plant species and their communities, it is unclear how important allelopathy is as a characteristic of many invaders as opposed to a few well-studied examples. In other words, the degree to which allelopathy is a key characteristic in the toolkit that boosts the success of exotic invasions is still unknown.

Brassica plants, including species like cabbages, broccoli, cauliflower, kale, and Brussels sprouts, contain allelochemical compounds like glucosinolates. These compounds, under exceptional conditions, can be released into the environment and have been observed to affect seed germination and plant growth (Bones and Rossiter 1996).

*Hirschfeldia incana*, commonly known as shortpod mustard, is closely related to the Brassica genus and belongs to the Brassicaceae family, often referred to as the mustard family. This family includes a wide range of well-known vegetables and oilseed plants. The relationship between *Hirschfeldia incana* and Brassica species is characterized by their genetic, morphological, and ecological similarities. These similarities include the production of glucosinolates (sulfur-containing compounds), four-petaled flowers arranged in a cross shape, and seed pods known as siliques.

The taxonomy of the Brassicaceae family is complex and subject to revisions as new genetic information becomes available. The close relationship between *Hirschfeldia incana* and Brassica species is not only evident in their physical appearance but also supported by molecular studies that examine DNA sequences to understand their evolutionary relationships.

Furthermore, *Hirschfeldia incana*'s ability to thrive in disturbed soils and its widespread distribution as a weed can provide valuable insights into adaptability and ecological strategies shared with some Brassica species. These Brassica species are known for their capacity to grow in various environmental conditions. Understanding these relationships has significant implications for agriculture and horticulture, as it can aid in the development of more resilient crop varieties.

This research has many benefits, one of them is being able to identify another plant species that has the potential to suppress the growth of an invasive species. Also over the course of time researchers might discover a native plant that might inhibit the growth of the invasive species. It can also open some doors for scientists' ideas like how to control allelopathy. Also it would show us if there is a significant characteristic that both plants share. In this research, we delve into the sample collection and preparation methods employed for shortpod mustard, a species with potential allelopathic properties, and provide insights into how similar procedures can be adapted for studying sunflowers (*Helianthus annuus*). These distinct plant species offer valuable insights into the world of plant ecology, allelopathy, and ecological interactions, shedding light on the intricate relationships that exist within ecosystems.

The main goal of the study was to see how the liquid from shortpod mustard leaves affects the growth of sunflower seeds. We did this by comparing how many seeds sprouted in two different petri dishes. One dish had plain water (our control group), and the other had the mustard leaf liquid. By looking at the differences in how many seeds grew in each dish, we could understand the effect of the mustard leaves on the sunflower seeds.

## METHODS

### STUDY SPECIES

Shortpod mustard (*Hirschfeldia incana*) and sunflower (*Helianthus annuus*) are two plant species that have captured the attention of ecologists and botanists alike due to their distinct characteristics and significant ecological roles. Each of these plants possesses unique traits and ecological significance, making them compelling subjects of study in the realm of plant science.

*Hirschfeldia incana*, commonly known as shortpod mustard, is a remarkably resilient plant species with allelopathic properties that have piqued the interest of researchers. This member of the Brassicaceae family thrives in a variety of environments, including disturbed ecosystems like roadside verges, agricultural fields, and other areas with disrupted natural habitats. What sets shortpod mustard apart is its ability to release biochemical compounds into its surroundings, thus potentially influencing the growth and development of neighboring plants. Understanding the allelopathic interactions of shortpod mustard, as well as the chemical constituents responsible for these effects, holds profound ecological importance. This knowledge can shed light on its ecological impact and uncover potential applications in areas such as weed management and sustainable agriculture.

On the other hand, *Helianthus annuus*, known as the common sunflower, boasts its own distinctive characteristics and ecological significance. Belonging to the Asteraceae family, sunflowers are easily recognizable by their vibrant yellow flowers and towering stalks, making them iconic in the botanical world. Beyond their aesthetic appeal, sunflowers serve multiple

practical purposes, including the production of edible seeds and oil. However, their ecological role extends beyond human consumption. Sunflowers are renowned for their competitive growth and allelopathic potential, which can influence neighboring plant species and ecosystem dynamics. Thus, delving into the study of sunflowers offers valuable insights into their ecological interactions, growth patterns, and potential impacts on surrounding vegetation.

In essence, shortpod mustard and sunflower, with their contrasting yet complementary attributes, form a captivating duo for ecological research. By unraveling the mysteries of their allelopathic interactions and biochemical constituents, we gain a deeper understanding of their roles in the natural world and unlock potential applications that can benefit both science and society.

## **SAMPLE COLLECTION AND PREPARTION**

### *Shortpod leachate preparation*

Mature shortpod mustard leaves, known for their potential allelopathic properties, were collected during the early morning hours from the vicinity of Crest Plaza Riverside. Following the collection, to preserve the leaves, we used a freeze-drying method right after collection. This method, called freeze-drying or lyophilization, involved freezing the leaves at very low temperatures and reducing the pressure around them. This process removed moisture from the leaves while keeping their biochemical properties intact. Once the freeze-drying was done, we roughly split the dried leaves into 8 Eppendorf tubes and weighed the samples. The total weight of dried leaf tissue was 0.69 grams.

To initiate the extraction process, a combination of disruption beads and bead silica mobile was added to each sample. Then the samples were homogenized at a consistent speed of 1800 rpm for a precise duration of one minute. This was followed by the addition of a calculated 5.5 ml of water, distributed evenly across the 8 tubes (0.6875 ml/tube). These samples were mixed vigorously.

Once done, these samples were incubated on a calibrated heating block at 25°C for a 24-hour cycle, promoting maximum extraction. Following the incubation, a brief centrifugation process at high speed was applied for 10 seconds. The centrifuge was used to separate the heavier parts from the liquid. After this process, the clear liquid (leachate) was carefully moved into a single 15 ml tube, and more water was added to bring the total volume up to 6.9 mL (Figure 1). The leachate, now at a 1:10 dilution (0.69 grams of tissue in 6.9 mL of water), was ultimately stored in the larger tube and refrigerated for stability, ready for the next phase of the experimental procedure involving seed germination tests.

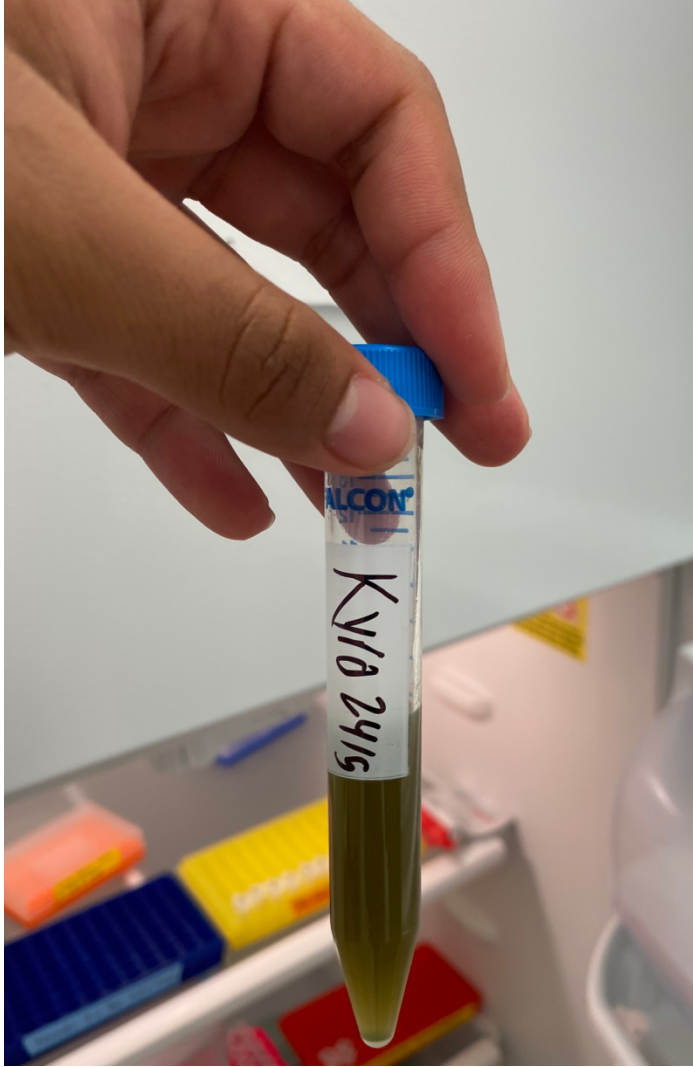


Figure 1: Final look of the leachate

*Sunflower seed preparation*

From the regions of Inland Empire, CA, 36 distinct populations of sunflower seeds, *Helianthus annuus*, were meticulously selected. These populations were represented by designations ranging from IES028 to IES528, providing a comprehensive overview of the diversity and ecological niche variations present in the region.

From each of these populations, a subset of 14 seeds was chosen. Prior to any treatment, it was of paramount importance to ensure the seeds were free from contaminants and in prime condition for the experiment. To this end, they were subjected to a sterilization regimen which began with a brief 10-second immersion in 70% ethanol. Following this, the seeds were washed twice in sterile water to rid them of any residual ethanol. After the cleaning process, the seeds underwent a scarification procedure, a critical step in which the seed coat is intentionally exposed or altered. This scarification process is essential to enhance the germination potential of the seeds by aiding in the breaking down of the seed coat barriers, allowing for the absorption of water and other essential nutrients required for successful germination. These seeds were then evenly distributed into two sterile petri dishes, denoted A and B, with each dish housing 5-7 seeds. This resulted in 72 total petri dishes (36 population x 2 treatments).

#### *Experimental treatment*

The actual testing phase involved the introduction of filter paper into each petri dish. The A dish, serving as a control, was treated with a measured 2.5 ml of distilled water. In contrast, the B dish, the test group, was saturated with 2.5 ml of the meticulously prepared shortpod mustard leaf leachate. These petri dishes were moved to a dark drawer for 7 days in order to allow the seeds to germinate.

#### **SCORING GERMINATION AND STATISTICAL ANALYSIS**

The evaluation of seed germination in this study involved a meticulous and structured procedure. Initial data collection included the recording of the total number of seeds placed in Petri Dish A and Petri Dish B, as well as the subsequent count of seeds that successfully germinated within

each dish. We considered seeds to be germinated if The presence of the white radicles is there this indicates that these seeds have begun the germination process.. This data formed the basis for assessing germination rates in both treatments.

Beyond the descriptive statistics, a rigorous statistical analysis was conducted to ascertain the significance of the observed differences in germination between Treatment A and Treatment B. This analysis took the form of a one-sample t-test. The null hypothesis, set as the true mean difference in germination percentages being equal to zero, was put to the test against the alternative hypothesis, which posited that the true mean difference was not equal to zero. In this study, an analysis of germination data from two distinct treatments (Treatment A and Treatment B) was carried out. Initially, the germination data was imported from a CSV file named "2023\_Seed\_Germination\_Results.csv" and stored in a variable denoted as data1. To further investigate the differences between the treatments, a new data frame labeled data2 was created. Within this data frame, two new columns, A\_percent and B\_percent, were calculated using the dplyr package, representing the germination percentages for Treatment A and Treatment B, respectively. Additionally, the difference between these percentages, referred to as diff, was computed to quantify any disparities between the treatments. Then a one-sample t-test was used to determine whether the mean of the differences was significantly different from zero.

## **RESULTS**

The output from the one-sample t-test provided critical insights into the significance of these observed differences. A t-value of 6.1991 was calculated, while the degrees of freedom (df) were

determined to be 35. However, it was the p-value of  $4.225e-07$  that held particular significance, indicating an exceedingly low probability of obtaining such results by random chance alone. This extremely small p-value led to the rejection of the null hypothesis. In practical terms, this implied that there was indeed a substantial and statistically significant difference in germination percentages between Treatment A and Treatment B. The t-test results not only signify a statistically significant difference in germination percentages but also clearly indicate that Treatment A (the control) led to a higher germination rate compared to Treatment B (the leachate treatment). This distinction is crucial as it suggests that the conditions or specific elements of Treatment A were more conducive to promoting seed germination. The direction of this effect is fundamental to our understanding of how different treatments can influence germination and provides a concrete basis for recommending Treatment A as the more effective method for enhancing germination in this context.

Furthermore, a 95 percent confidence interval was provided to shed light on the range within which the true mean difference in germination percentages was likely to fall. This interval offered valuable insights into the magnitude of the difference, aiding in the interpretation of the practical implications of the findings.

In summation, this comprehensive approach to scoring germination, coupled with the one-sample t-test and its associated results, provided a thorough and statistically validated assessment of the differences in seed germination rates between the two treatments, enhancing the reliability and depth of our research findings. In figure 2, seeds are situated on moist filter paper, with many exhibiting germination through visible roots and shoots. This indicates that Treatment A

provides a supportive environment for germination, likely serving as the control setup offering ideal growth conditions. In contrast, figure 3 shows seeds under the same moist conditions, yet with a significantly reduced germination rate, indicating that Treatment B, which includes leachate, has a detrimental effect on the seeds' ability to germinate. The comparison between the two images illustrates the varying influence that the treatments have on seed germination, with Treatment A being more favorable compared to Treatment B with its leachate application.



Figure 2 : sample from petri dish A

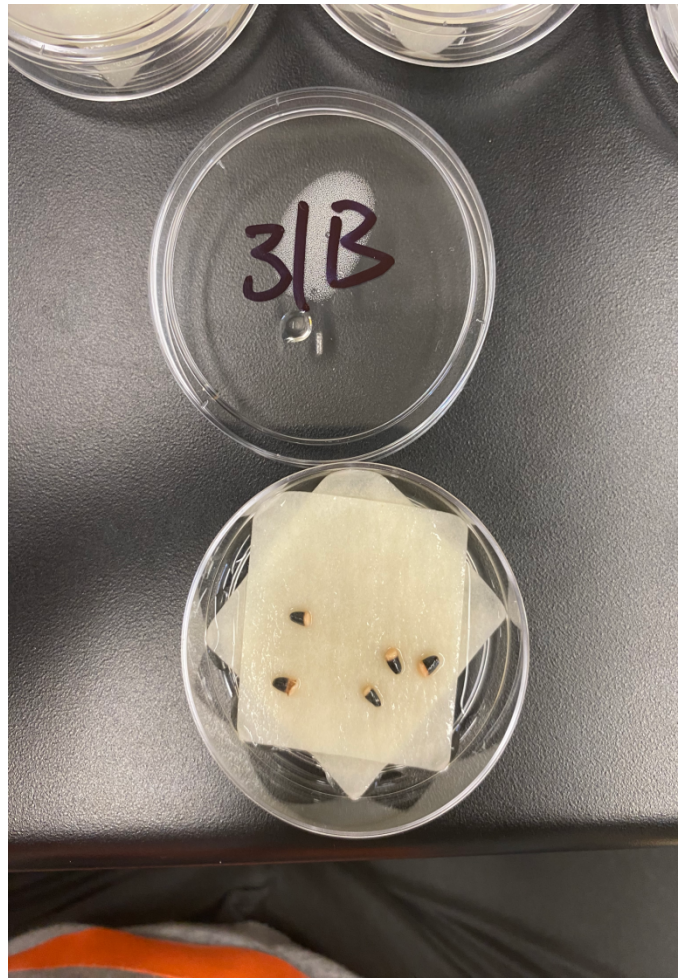


Figure 3 : sample from petri dish

Delving into Figure 3, which illustrates the distribution of these differences in germination percentages, we see a jitter plot enhanced by a boxplot. The jitter plot methodically spreads out individual data points to avoid overlap, making patterns within the data more apparent. This is particularly useful in illustrating the slight variability and density of data around the median. The accompanying boxplot succinctly encapsulates the interquartile range and median, providing a clear visual summary of the central tendency and dispersion of the data.

In the plot, the red dashed line at the level of  $y = 0$  serves as a critical benchmark, representing the hypothetical line of no difference. It is against this backdrop that the differences in germination percentages are evaluated. Most of the data points hover above this line, suggesting that the seeds in Treatment A generally had a higher germination rate compared to those in Treatment B.

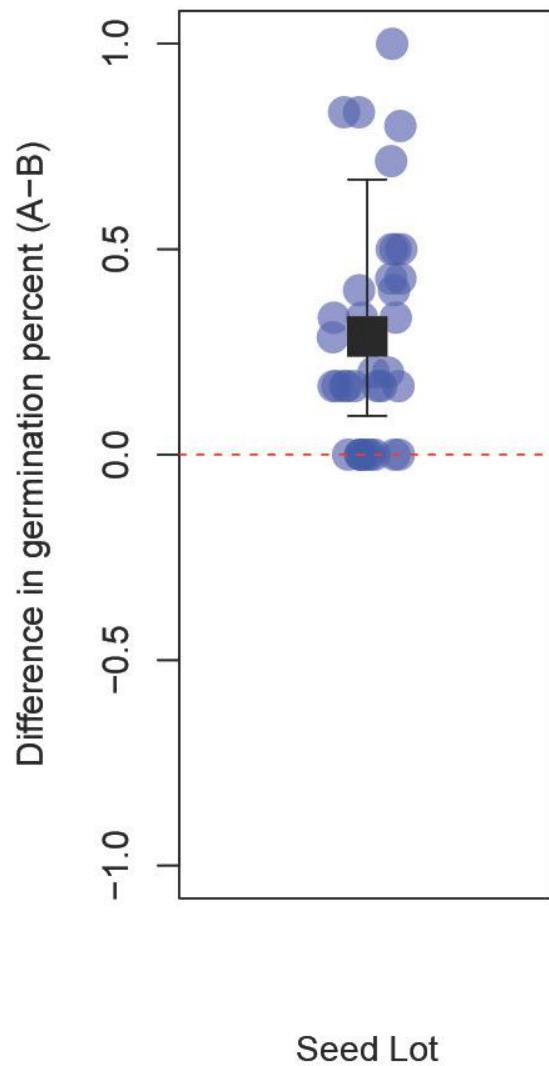


Figure 3: The figure

is a dot plot showing that

seed treatment A which had just water generally has a slightly higher germination percentage than treatment B which was treated with the leachate, with most of the differences clustered just above zero.

## **DISCUSSION**

Expanding upon our study of allelopathic effects between shortpod mustard and sunflower seeds, it becomes pertinent to explore the broader ecological and evolutionary implications of these interactions. One intriguing aspect to consider is the evolutionary arms race between plants, where species like shortpod mustard develop sophisticated chemical strategies to suppress competitors, which in turn may evolve resistance mechanisms. Investigating this dynamic could provide deep insights into the co-evolutionary processes shaping plant communities.

A further important area of study is the role allelopathy plays in influencing plant-soil interactions. The allelochemicals emitted by shortpod mustard could potentially modify the composition of the soil's microbial community. This alteration might impact not only sunflowers but also a variety of other plant species coexisting in the same environment. By exploring these interactions that are mediated through the soil, researchers can gain insights into the intricate multi-level relationships that exist within ecosystems. Such understanding is essential for informing practices aimed at maintaining and improving soil health, as it provides a clearer picture of how allelopathic plants interact with and affect their surrounding soil ecosystems.

In addition, the impact of allelopathy on pollinators and other non-target organisms is an area that warrants thorough investigation. While the focus of our study was on germination inhibition, allelochemicals might also affect the health and behavior of pollinators, potentially influencing plant reproductive success and ecosystem services like pollination.

Considering the impact of climate change on allelopathic interactions forms another critical research avenue. Climate factors such as temperature and precipitation can influence the production and efficacy of allelochemicals. Investigating how climate change might alter the balance of competitive interactions in plant communities could have significant implications for predicting future biodiversity patterns and ecosystem resilience.

Moreover, the potential of allelopathic plants in phytoremediation deserves attention. Plants like shortpod mustard, which can release potent chemicals into the soil, might be capable of aiding in the degradation or immobilization of soil pollutants. This application would not only contribute to environmental clean-up efforts but also add a new dimension to understanding the role of allelopathy in ecological restoration.

Exploring the genetic basis of allelopathic traits in plants like shortpod mustard could also offer valuable insights. Identifying the genes involved in the synthesis and release of allelochemicals could help in understanding the regulation of these traits and their variability across different environmental conditions.

Furthermore, the incorporation of allelopathic plants in agroforestry systems could be explored. These systems, which integrate trees and shrubs with crops and livestock, could benefit from the strategic use of allelopathic species to control weeds and enhance soil health, thereby reducing reliance on chemical herbicides.

Additionally, a comparative study of allelopathic effects across different plant families could reveal underlying patterns and mechanisms. Such a study would involve screening a range of species for allelopathic properties and comparing their effects on a common set of test species. This could lead to a broader understanding of the ecological roles of allelopathy and its evolutionary origins. Expanding this research could also uncover how allelopathy influences plant competition and survival, potentially offering new insights into ecological adaptation and species resilience in varying environmental conditions.

Finally, exploring the potential of allelopathic compounds in medicine could open new doors in pharmacology. Many plant-derived compounds have medicinal properties, and those involved in allelopathy might have unexplored therapeutic uses.

In conclusion, while our initial study offers valuable insights into allelopathic interactions between shortpod mustard and sunflower seeds, the field is ripe with opportunities for further exploration. Future research in these areas could significantly enhance our understanding of allelopathy's role in ecology, evolution, agriculture, and beyond, contributing to a more sustainable and holistic approach to managing our natural resources.

## CONCLUSION

In conclusion, our research on the allelopathic interactions between shortpod mustard and sunflower seeds opens up exciting avenues for future exploration. One critical area of interest is the isolation and characterization of specific chemical compounds within shortpod mustard leachate responsible for the observed inhibitory effects. This endeavor could pave the way for the development of targeted, eco-friendly herbicides, revolutionizing weed management practices. Moreover, extending our study to encompass a broader spectrum of plant species and delving into the influence of environmental variables on allelopathy promises to provide a more holistic understanding of this ecological phenomenon. Additionally, investigating synergistic relationships between allelochemicals and other ecological processes holds the potential to uncover novel insights into the dynamics of plant communities and ecosystems. Lastly, the practical applications of our findings in restoration ecology, particularly in the control of invasive species and the restoration of native plant communities, offer tangible solutions to environmental challenges in diverse landscapes.

In summary, the insights gained from our investigation into the allelopathic effects of shortpod mustard on sunflower seed germination provide a foundation for a wide range of future research endeavors. These studies have the potential to significantly advance our understanding of ecological interactions, contribute to sustainable agricultural practices, and aid in the conservation and restoration of natural plant communities. The intersection of ecological research and practical application holds great promise for addressing some of the pressing environmental challenges we face today.

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