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Allelopathic Effects of *Ageratum conyzoides* Root Exudates on Germinability of Selected Crops: A Comparative Analysis

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Abstract: This study investigates the allelopathic effects of aqueous root exudate extracts of *Ageratum conyzoides* on the germination, shoot length, and root length of various agricultural crops. The study reveals how different species respond uniquely to varying concentrations, uncovering complex dynamics in crop-allelopathic interactions. The germination analysis reveals distinct sensitivities among the tested crops. *C. sativus* demonstrates resilience, displaying minimal germination reduction even at higher exudate concentrations. *C. arietinum* exhibits moderate susceptibility, while *Z. mays* emerge as the most sensitive crop, displaying a profound reduction in germination. *L. esculentum* and *P. vulgaris* also exhibit sensitivity, with adverse impacts on germination rates. *A. esculentus* consistently shows reduced germination rates with increasing exudate concentrations. Shoot length dynamics show that all crops experience greatest elongation without *A. conyzoides* root exudates, suggesting inhibition of growth by these exudates. Shoot growth declines with higher exudate concentrations across most crops, with varying sensitivities. *C. sativus* consistently decreases, while *C. arietinum*, *Z. mays*, and *P. vulgaris* show concentration-dependent inhibition. *S. lycopersicum* significantly reduces shoot elongation, and *A. esculentus* remains consistently sensitive. Root length patterns further elucidate the allelopathic effects. Responses vary across species and concentrations. *A. esculentus* roots thrive at lower concentrations but decline at higher ones. *S. lycopersicum* shows initial reduction, significant decline, followed by recovery. *Z. mays* and *C. arietinum* exhibit fluctuating responses. *P. vulgaris* consistently grows, while *C. sativus* persistently displays reduced root elongation. These findings highlight the complex and species-specific allelopathic interactions between *A. conyzoides* and agricultural crops, emphasizing the need for a comprehensive understanding of these dynamics for effective crop management in *A. conyzoides*-infested areas.

Keywords: Allelopathy, Root exudates, Agricultural crops, Seed germination, Ageratum conyzoides.

RESEARCH PAPER

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INTRODUCTION

The uncontrolled expansion of agriculture across nations, often neglecting the inadvertent introduction of accompanying weeds during the import and export of plant materials, has emerged as a key driver behind the escalating challenges associated with weed management in crop production. These weeds, particularly allelopathic ones, pose a significant threat to both biodiversity and agricultural enterprises. One of the invasive weeds, such as *Ageratum conyzoides*, commonly known as billygoat weed, is an erect (30-80 cm), herbaceous annual weed is native to tropical America but has spread widely, including in South-East Asia, particularly hilly areas (Kaul and Neelangingi, 1989: Ray *et al.*, 2019). While it thrives in cultivated lands, it can also adapt to diverse habitats such as grasslands, wastelands, and vacant areas (Okunade 2002, Batish *et al.*, 2009: Juliana *et al.*, 2010). *A. conyzoides* poses a significant challenge to farmers, causing substantial economic losses by interfering with the growth of both summer and winter crops (Bhatt *et al.*, 2001). Some farmers have even been compelled to abandon their fields due to its infestation. Furthermore, in rangelands, this weed disrupts the availability of native grasses, leading to fodder scarcity (Koli *et al.*, 2006). The dominance of *A. conyzoides* over other vegetation hints at an interference mechanism which is known as allelopathy, involves the release of chemicals by living or dead plant parts, often with negative effects on associated plants (Osman *et al.*, 2021; Haq *et al.*, 2020; Kaur *et al.*, 2012). This strategy is frequently adopted by invasive weeds to establish themselves successfully in an area, as reported in 240 weed species by Qasem and Foy (2001). Hafsah *et al.* (2012) found that *A. conyzoides* is a dominant weed in agricultural fields, hindering the growth of cultivated plants due to its allelopathic capabilities. Chemical exudates from this weed disrupt neighboring plant growth and ecological balance. The leaves of *A. conyzoides* contain phenolic acids like gallic acid, coumaric acid, and proteocatechins acid, which can inhibit the growth of certain plant species. Notably, the allelopathic potential of this weed leaves has been ascertained, owing to the presence of three key phenolic acids: gallic acid, coumaric acid, and proteocatechins acid. These phenolic compounds, falling within the phenol group, possess the ability to impede the growth of specific plant species (Fatema, 2013). Aqueous extracts, volatile oils, and rhizospheric soil of *A. conyzoides* contain allelochemicals that disrupt nearby plants (Kong *et. al.*, 2002, Sing *et. al*., 2003). It affects germination, plumule and radicle length of various crops (Idu *et al*., 2013). Accumulated weed residue in infested areas also hinders subsequent crop growth [40]. Allelochemicals are released via leachates, volatilization, and decomposition of organic matter. They impact respiratory enzymes (Muscolo *et al*., 2001) and gibberellic acid, vital for seed germination (Olofsdotter, 2001). Allelochemicals trigger oxidative stress in plants by generating Reactive Oxygen Species (ROS), extremely reactive free radicals. While ROS are essential for regular biological processes and signaling pathways, an excess can damage tissues and disrupt plant functions. ROS inflict harm primarily through three mechanisms: lipid peroxidation within cell membranes and cytosol, DNA damage resulting in mutations and cell death, and protein modification via oxidation (Eberhardt 2001).

The use of herbicides presents a myriad of issues, including the development of resistance in organisms, environmental pollution, and potential toxicity-related health hazards in both humans and livestock (Cailati *et al*., 2020). These practices are clearly unsustainable and cannot be perpetuated indefinitely. Prior to the widespread adoption of herbicides, weed control primarily relied on cultivation techniques and crop rotation (Weisberger, 2024). Furthermore, the utilization of allelopathy emerges as a prudent and secure alternative to address these pressing concerns. Allelopathic strategies offer a promising avenue to tackle these problems and promote sustainability in agriculture while safeguarding the environment for future generations (Shretha *et al*., 2021). This approach aims to mitigate environmental pollution and uphold ecological equilibrium, particularly concerning soil fauna and flora (Lago-Olveira, 2024). It achieves this by reducing the reliance on chemical herbicides or substituting them with naturally derived products (Weisberger, 2024). However, reintroducing such beneficial allelopathic traits could be feasible through conventional breeding and modern molecular-genetic techniques (Ain, 2023). The strategic use of allelochemicals in agriculture could be pivotal in managing parasitic weed problems. These compounds can either inhibit or stimulate seed germination, providing targeted control. The using natural stimulants like strigol and sorgolactone from various plants for managing parasitic weeds (Macias, 2019). The using catch-and-trap crops for integrated weed management, leveraging the allelopathy mechanism to control parasitic species. Weeds have historically been viewed as a nuisance or unwanted plants, with definitions evolving to consider their ecological impact. In modern agriculture, allelopathic crops are employed as cover crops, intercropping systems, green manure, and crop rotations (Khamare, 2022). This study investigates the allelopathic effects of H. indicum on various cucurbit crops, focusing on the impact of leaf and root extracts on seed germination and subsequent plant growth.

MATERIALS AND METHODS

Collection of Plant Materials:

To evaluate the allelopathic effect of some weeds root exudate on germinability and biomass production of crop seedlings, seeds of crop species v. z. *A. esculentus, S. lycopersicum, P. vulgaris, Z. mays, C. arietinum, C. sativum.* were collected from local bazar, hathazari, Chittagong university campus. *A. conyzoides* at flowing stage were collected as donor plant from the botanical garden within the Department of Botany, University of Chittagong, Chittagong, Bangladesh.

Preparation of Root Exudates:

Approximately 40 weed species were manually removed from crop fields by uprooting them from the soil. To ensure the cleanliness of the weed roots, they were precisely washed first with tap water and then with distilled water, eliminating any potential contaminants. Following this, the weed plant roots were promptly immersed in a conical flask, which was covered with foil paper, containing 1200 ml of distilled water. The aerial parts of the plants were exposed to sunlight for a period of five hours to stimulate the exudation of compounds from the roots.

Subsequently, the resulting root exudate was methodically filtered using Whatman No. 1 filter paper, resulting in a clear liquid. The filtrates were then divided into three equal aliquots, with each aliquot containing 400 ml of the solution. The initial aliquot was denoted as T1, while the second aliquot underwent a concentration process, reducing its original volume by 50%, and was consequently labeled as T2. The final aliquot underwent further concentration, resulting in a volume of 125 ml, representing approximately 25% of the original volume, and was designated as T3. This systematic partitioning of the filtrates facilitated detailed experimentation and analysis based on the relative percentages of the original solution. Root exudates of *A. conyzoides* were collected using a non-destructive method by immersing the roots in distilled water for a specific duration.

Bioassay:

Seeds were carefully selected based on their uniform size, shape, color, and overall health. They were then subjected to sterilization in 70% ethanol for a duration of 1-2 minutes, followed by a thorough rinse with sterile distilled water repeated five times to remove any residual chemicals.In the soil experiment, Petri dishes were filled with garden soil, and ten pre-treated seeds were planted and irrigated with 5 mL of the respective aqueous root exudates. The control group received an equivalent amount of water. The growth chamber was maintained at a constant temperature of 25°C, and moisture levels in the Petri dishes and other containers were carefully monitored. Seed germination was considered to have occurred when the radicle length exceeded 2 mm. After a 10-day incubation period, various biochemical indicators were assessed, including the number of germinated seeds, root and shoot lengths, as well as the seedlings' overall weight.

Data Analysis

The data underwent three rounds of analysis using MS Excel 2010. All results are presented as mean values accompanied by their respective standard Error of Mean (SEM).

RESULTS AND DISCUSSION

Alterations in Seed Germination:

The radar chart depicted in Figure 1 provides a comprehensive illustration of the intricate dynamics underlying the influence of *A. conyzoides* root exudates on the germination processes of various agricultural crops (Figure 2). Remarkably, each crop exhibits a distinct response profile to the allelochemicals, thereby elucidating the nuanced interplay between *A. conyzoides* and these crops. Intriguingly, the data reveals that *C. sativus*, in contrast to its counterparts, exhibits a notable resilience to the allelopathic compounds present in *A. conyzoides.* This resilience is exemplified by the marginal decline in germination rates from T_0 to T_3, signifying the cucumber's potential to withstand the inhibitory effects of these exudates, even at higher concentrations (Hafsa *et al*., 2012). This observation suggests that *C. sativus* cultivation may be less adversely affected when coexisting with *A. conyzoides.* Conversely, the *C. arietinum* displays a more moderate reaction, characterized by a discernible reduction in germination rates, particularly pronounced at T_2 and T_3, indicating a moderate susceptibility to the allelopathic substances. This implies that while *C. arietinum* may not be as robust as *C. sativus* in the presence of *A. conyzoides*, it may still sustain a certain degree of productivity, albeit with some compromise. Therefore, incorporating *C. arietinum* into a crop rotation strategy alongside *A. conyzoides* necessitates a cautious consideration of this susceptibility. Contrastingly, the corn emerges as the crop most vulnerable to the allelochemicals, as even the lowest concentration of root exudates at T_1 results in a substantial reduction in germination, with a further exacerbation as concentrations escalate. This profound sensitivity gives emphasis to the considerable impact that *A. conyzoide*s could exert on *Z. mays* cultivation, rendering it a particularly precarious endeavor in fields previously inhabited by the weed. *S. lycopersicum,* like *Z. mays,* exhibits a noteworthy sensitivity to the root exudates, with a pronounced decline evident at T_1, followed by a progressively adverse impact at T_2 and T_3. This heightened sensitivity raises concerns regarding the feasibility of *S. lycopersicum* planting in fields where *A. conyzoides* has previously thrived, as it is susceptible to substantial germination impediments (Wardani *et al.*, 2018). Similarly, the *P. vulgaris* displays a dose-dependent sensitivity, with each incremental increase in exudate concentration correlating with a decline in germination rates. Although not as severely affected as *Z. mays,* the significant impact on *P. vulgaris* germination warrants careful consideration when devising crop management strategies. Lastly, the *A. esculentus* demonstrates a consistent reduction in germination rates as exudate concentrations rise, indicating a sustained sensitivity to the allelochemicals (Abbas *et al.*, 2017, Motmainna *et al*., 2021). This observation suggests that *A. esculentus*, like other crops, may experience adverse effects in the presence of A. conyzoides. Consequently, this sensitivity should be factored into intercropping or successive planting schemes involving *A. conyzoides* to ensure optimal crop performance and yield. The critical discussion of these results indicates a species-specific allelopathic effect of *A. conyzoides* root exudates on crop germination (Akter, 2024).

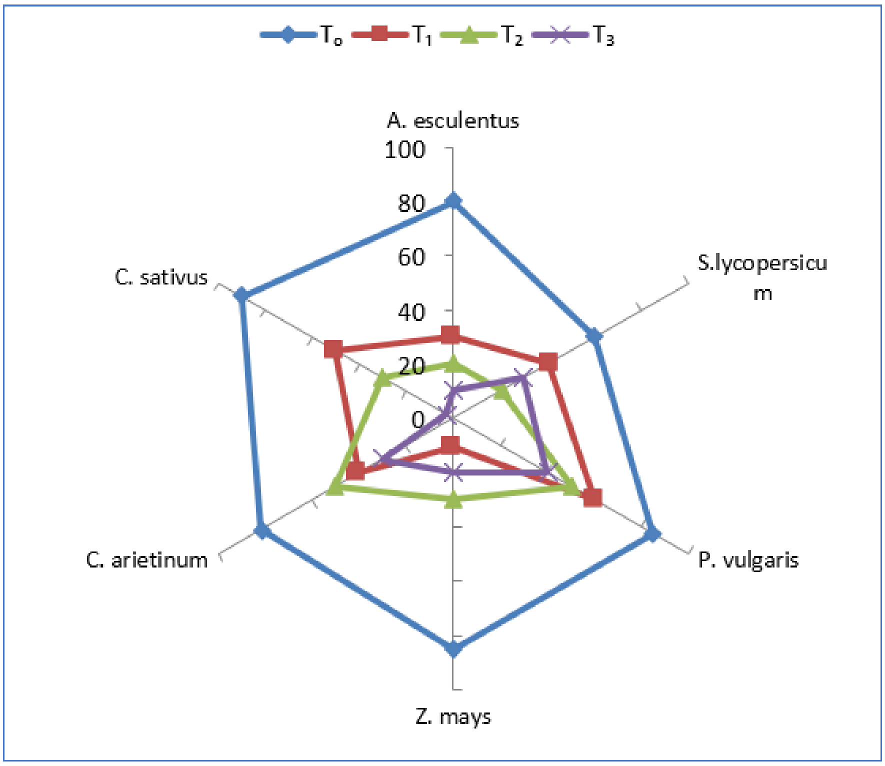

Figure 1: Radar chart showing the % of seed Germination of six agricultural crops to distilled water (T_o) and different concentrations of Ageratum conyzoides root exudates (T_1- T_3).

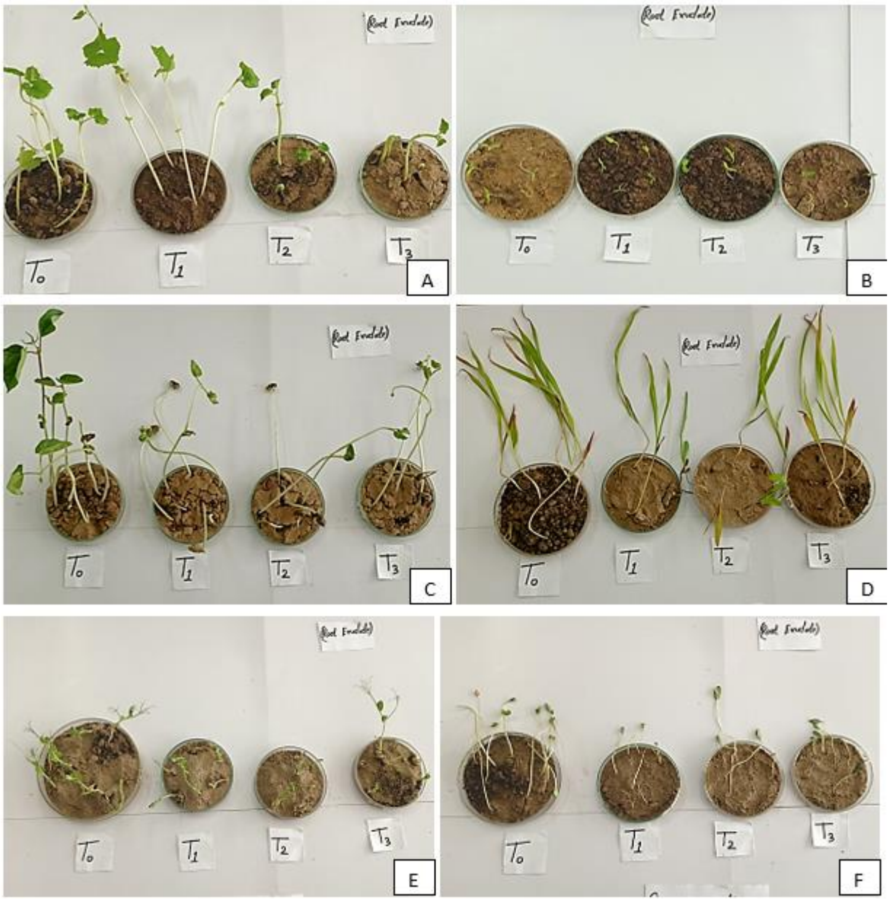

Figure 2: Treatment of aqueous (T_1- T_3) root exudate extracts of the *of A. conyzoides* on the germination of six vegetable crops (A-F) at 10 days. A (*Abelmoschus esculentus*), B (*Solanum lycopersicum*), C. (*Phaseolus vulgaris*), D (*Zea mays*), E (*Cicer arietinum*), F (*Cucumis sativus*)

Shoot Length Dynamics

The results presented in Table 1 showed the highest shoot elongation across all crops in control treatment, suggesting that the root exudates may have an inhibitory effect on growth. For *A. esculentus and S. lycopersicum*, the shoot elongation decreased with increasing concentration of root exudates (T1-T3), indicating a concentration-dependent inhibition (Zohaib *et al*., 2017, Akter, 2023). *P. vulgaris* showed a less clear pattern with T1 being lower than T0 but T2 showing a slight increase compared to T1, followed by a decrease at T3. In *Z. mays* and *C. arietinum*, the shoot length was reduced at T1 and then showed a further decrease at T2, but less of a decrease at T3, suggesting a potential threshold effect where beyond a certain concentration (Jabeen *et.al.*, 2009), the additional inhibitory effect is minimized. Finally, *C. sativus* exhibited a consistent decrease in shoot elongation with increasing exudate concentration, although the reduction from T2 to T3 was less pronounced than from T0 to T1 and T1 to T2 (Figure 3).

Table 1: Shoot elongation of receptor agricultural crops to distilled water (T_o) and different concentrations of *Ageratum conyzoides* root exudates (T_1- T_3) extracts at 10 days (Mean±SEM)

Treatment	*A. esculentus*	*S. lycopersicum*	*P. vulgaris*	*Z. mays*	*C. arietinum*	*C. sativus*
T_o	19.1±0.08	6.33±0.16	32.23±0.21	8.13±0.12	11.3±0.6	12.17±0.12
T_1	13.47±0.12	4.1±0.08	28.13±0.12	7.3±0.16	5.3±0.22	5.8±0.08
T_2	15.37±0.26	3.67±0.16	24.36±0.12	7.23±0.17	6.27±0.1	10.2±0.16
T_3	10.33±0.17	3.23±0.21	20.23±0.21	10.5±0.25	13.33±0.25	11.23±0.21

The observed trend in the result suggests that *A. conyzoides* root exudates have an allelopathic effect on the shoot elongation of various agricultural crops. This inhibition of growth aligns with the allelopathy theory, where compounds produced by one plant can adversely affect the growth of neighboring plants (Koocheki, 2013). The consistent decrease in shoot elongation for *A. esculentus, S. lycopersicum*, and *C. sativus* with increasing concentrations of root exudates strongly supports this hypothesis. The variations in sensitivity among the different species indicate that the allelochemicals present in the *A. conyzoides* exudates might be affecting multiple physiological pathways. For instance, the relatively stable decrease in *Z. mays and C. arietinum* could suggest a saturation point beyond which no further inhibition occurs, indicating a possible threshold in the responsiveness of these plants to the allelochemicals (Kobayashi 2004, Khan, 2019).

Root Length Patterns

The data from Table 2 elucidates the variable allelopathic effects of *A. conyzoides* root exudates on the root elongation of diverse agricultural crops. Contrary to the uniform inhibitory trend observed in shoot elongation, root elongation responses to the exudates were species-specific and varied across different concentrations (Figure 3). For *A. esculentus*, an initial promotion of root elongation was observed at the lowest exudate concentration (T1), which could be indicative of a hormetic effect (Duke *et al*., 2006)). Hormesis in allelopathy is characterized by low-dose stimulation and high-dose inhibition. However, subsequent concentrations (T2 and T3) led to a decline in root growth, consistent with the expected inhibitory allelopathic response (Bieberich *et al.*, 2018).

Table 2: Root elongation of receptor agricultural crops to distilled water (T_o) and different concentrations of *Ageratum conyzoides* root exudates (T_1- T_3) extracts at 10 days (Mean±SEM)

Treatment	*A. esculentus*	*S. lycopersicum*	*P. vulgaris*	*Z. mays*	*C. arietinum*	*C. sativus*
T_o	6.1±0.08	2.13±0.12	12.1±0.08	14.3±0.16	11.2±0.16	5.1±0.08
T_1	8.1±0.08	5.4±0.08	6.4±0.08	14.73±0.17	14.2±0.16	2.3±0.16
T_2	4.4±0.08	1.43±0.12	5.4±0.21	10.27±0.17	10.2±0.16	3.47±0.12
T_3	5.4±0.08	5.17±0.16	13.2±0.21	20.17±0.17	22.2±0.16	4.2±0.16

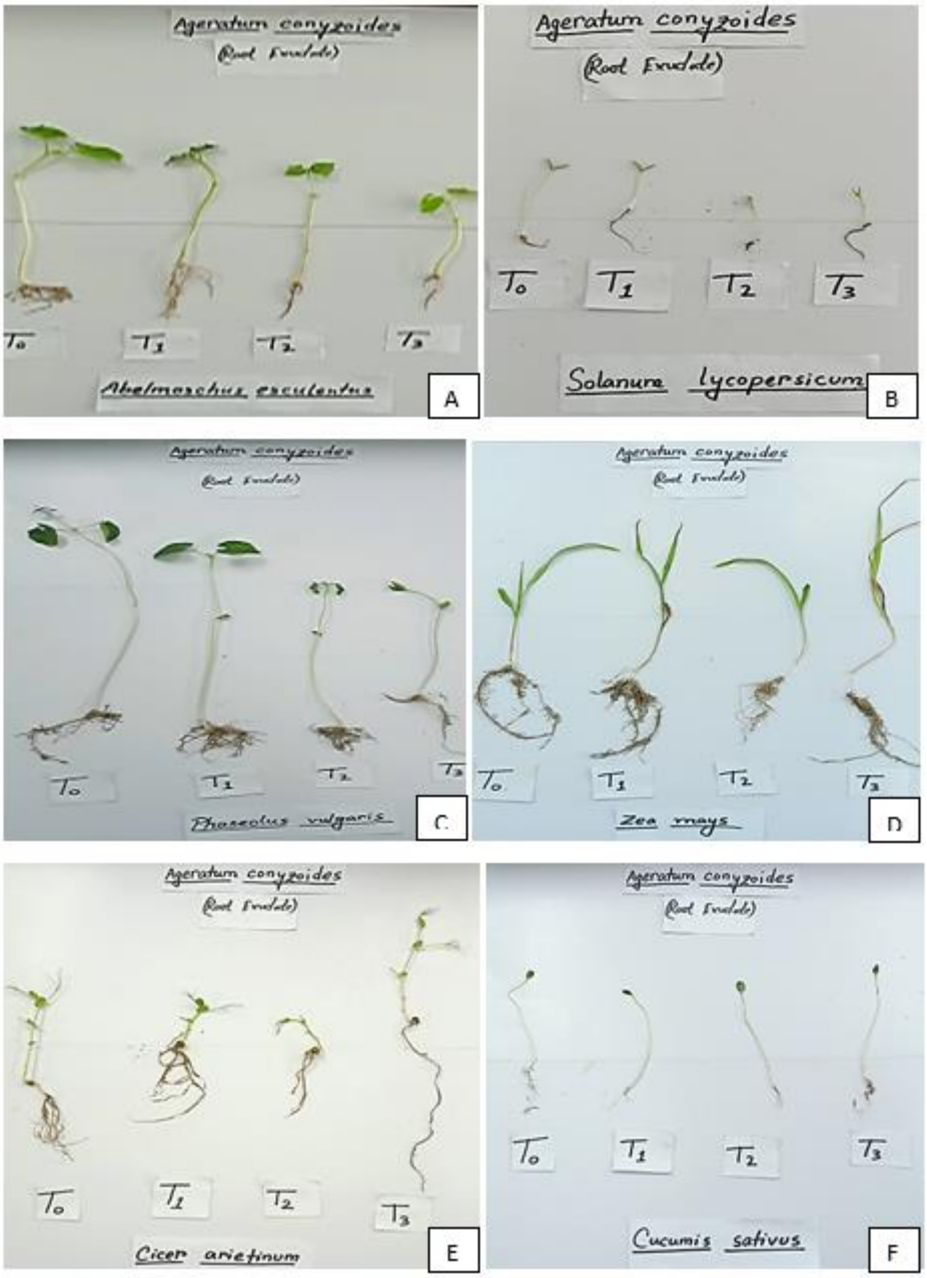

Figure 3: Treatment of aqueous (T_1- T_3) root exudate extracts of the *of A. conyzoides* on the Shoot and root length of six vegetable crops (A-F) at 10 days. A (*Abelmoschus esculentus*), B (*Solanum lycopersicum*), C. (*Phaseolus vulgaris*), D (*Zea mays*), E (*Cicer arietinum)*, F (*Cucumis sativus)*

The root elongation pattern of *S. lycopersicum* suggests a complex interaction with the exudates. The observed decrease at T1 followed by a significant reduction at T2 and a rebound at T3 might reflect an adaptive physiological response, potentially involving mechanisms such as enhanced efflux of toxins, induction of detoxification pathways, or alteration in root architecture to mitigate the effects of higher allelochemical concentrations (Kong *et al*., 2002, Sing *et al*., 2003). In contrast, *P. vulgaris* exhibited an increase in root elongation across all exudate concentrations. This unique response may suggest that the compounds present in *A. conyzoides* root exudates act as growth stimulants at the tested concentrations for this particular species, or it could indicate the key resistance to the allelochemicals. *Z. mays* displayed a biphasic response (Nweke *et al*., 2017) with initial stimulation at T1, followed by inhibition at higher concentrations (T2 and T3), which is characteristic of a typical allelopathic dose-response curv e. This response might be due to allelochemical-induced modifications in nutrient uptake or hormonal balance at low concentrations, with toxicity effects prevailing as the concentration increases. *C. arietinum* showed a marginal increase in root elongation at T1, leveling off at T2, and a slight decrease at T3. This pattern might indicate a threshold concentration for allelochemical toxicity or a saturation point beyond which root growth is not further inhibited. Finally, *C. sativus* demonstrated consistent sensitivity to the root exudates with a decrease in root elongation at all concentrations. This suggests that *C. sativus* may lack effective mechanisms to cope with the allelochemicals present in the exudates, leading to a steady decline in root growth (Scavo *et al*., 2019, Godlewska *et al*., 2021).

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study highlights the intricate and species-specific allelopathic interactions between *A. conyzoides* and various agricultural crops. The research reveals distinct sensitivities in terms of germination, shoot length, and root length among the tested crops, emphasizing the complexity of allelopathic relationships in agroecosystems. Notably, *C. sativus* displays resilience, while *Z. mays* appears highly sensitive. These findings underscore the need for tailored crop management strategies when dealing with *A. conyzoides*-infested areas.

For future recommendations, it is crucial to delve deeper into the underlying mechanisms governing these allelopathic effects. Further research should investigate specific chemical compounds responsible for these interactions and explore potential mitigation strategies to minimize crop losses. Additionally, long-term studies on the cumulative effects of *A. conyzoides* allelopathy on soil health and microbial communities would provide valuable insights for sustainable agricultural practices in weed-infested regions.

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