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Ecophysiological and biochemical analysis of *Pisum sativum* under combined allelopathic stress from *Lantana camara* and *Cassia tora*

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Abstract
The present study investigates the combined allelopathic effects of two invasive species, *Lantana camara* and *Cassia tora*, on the ecophysiological and biochemical responses of *Pisum sativum* (pea), a key pulse crop with significant agricultural importance. Allelopathy, involving the release of bioactive compounds by plants, poses a critical threat to crop growth and sustainability, particularly in regions affected by invasive species. In this controlled greenhouse experiment, aqueous leaf extracts of *Lantana camara* and *Cassia tora* were applied individually and in combination at varying concentrations to assess their impact on seed germination, growth parameters, photosynthetic pigments, and biochemical stress markers in Pisum sativum. The study revealed that dual exposure to the extracts resulted in a significant decline in germination percentage, root and shoot length, biomass accumulation, and chlorophyll content. Concurrently, stress-related biochemical markers such as proline, malondialdehyde (MDA), and antioxidant enzyme activities (catalase, peroxidase, and superoxide dismutase) increased markedly, indicating oxidative stress and a defensive biochemical response. Statistical analyses, including ANOVA and correlation, confirmed the severity of the combined allelopathic stress compared to individual treatments. The findings highlight the synergistic phytotoxic effects of invasive species and underscore the need for integrated weed management strategies in sustainable pulse-based agroecosystems. This research contributes to the broader understanding of invasion biology and provides practical implications for ecological crop management under the threat of multiple invasive weeds.

Keywords: Allelopathy, lantana camara, *cassia tora*, pisum sativum, invasive species, ecophysiology, biochemical stress markers, antioxidant enzymes, oxidative stress, sustainable agriculture, weed management, pulse crops, proline, malondialdehyde (MDA), chlorophyll degradation

Introduction

The increasing invasion of exotic plant species such as *Lantana camara* and *Cassia tora* in agricultural ecosystems has emerged as a major ecological concern due to their potential allelopathic effects on native and cultivated plant species. Allelopathy refers to the chemical inhibition of one plant species by another through the release of bioactive compounds, which can adversely affect seed germination, plant growth, survival, and physiological processes. *Pisum sativum* (pea), a vital pulse crop rich in protein and nutrients, is particularly sensitive to abiotic and biotic stresses during its early developmental stages. The current study focuses on the ecophysiological and biochemical responses of *Pisum sativum* under combined allelopathic stress exerted by *Lantana camara* and *Cassia tora*, two prolific invasive species known to release a wide array of secondary metabolites into the soil environment. These compounds, such as phenolics, flavonoids, and terpenoids, can significantly alter nutrient uptake, photosynthetic efficiency, antioxidant enzyme activity, and other vital physiological processes in recipient crops. When acting in combination, the allelochemicals from both invasive species may exert synergistic or antagonistic effects, further complicating the interaction dynamics in affected ecosystems.

This study is particularly important given the widespread distribution of *Lantana camara* and *Cassia tora* in tropical and subtropical agricultural zones, where they commonly coexist and compete with cultivated plants. Despite numerous studies focusing on individual allelopathic impacts of these species, limited attention has been given to their combined effects, which could be more detrimental due to potential interactions between their bioactive compounds. Understanding these complex interactions is essential for developing sustainable crop management strategies. The study adopts an integrative approach, analyzing both ecophysiological parameters such as germination rate, root and shoot length, biomass accumulation, and survival percentage, as well as biochemical markers including chlorophyll content, protein levels, and the activity of antioxidant enzymes like catalase, peroxidase, and superoxide dismutase. These markers serve as indicators of stress perception and adaptation mechanisms in *Pisum sativum* when exposed to allelopathic interference.

Moreover, the research sheds light on how combined allelopathic stress alters plant metabolism and affects the plant's energy allocation for growth versus defense. Stress-induced biochemical changes often involve oxidative stress, leading to the accumulation of reactive oxygen species (ROS), which in turn activates the antioxidant defense system. Therefore, measuring enzyme activities becomes crucial in understanding the plant's resilience and adaptive responses. The findings from this research not only contribute to the growing body of knowledge in invasion biology and crop ecophysiology but also provide practical insights for farmers and agronomists to make informed decisions regarding weed control and crop placement. As the world moves toward sustainable agriculture, identifying the ecological risks posed by invasive species and their biochemical interactions with crop plants becomes increasingly critical. This study provides a foundation for further research in allelopathy-mediated crop suppression and the development of biologically informed strategies to mitigate these impacts in pulse-dominated agroecosystems.

Background

Allelopathy, the process by which plants release biochemicals (allelochemicals) into the environment to influence the growth, survival, and reproduction of neighboring organisms, plays a crucial role in shaping plant interactions within agroecosystems. It can significantly affect crop productivity by either suppressing weeds or, conversely, by allowing invasive species to outcompete native or cultivated plants. Among the most concerning invasive species with strong allelopathic properties are *Lantana camara* and *Cassia tora*. *Lantana camara*, a woody shrub, has been widely recognized for its aggressive spread and its ability to release potent allelochemicals that inhibit germination and growth of surrounding flora. Similarly, *Cassia tora*, a fast-growing herbaceous plant, has shown the potential to interfere with crop development through its allelopathic actions. These invasive species pose a substantial threat to agricultural sustainability, particularly in pulse-growing regions. In this context, *Pisum sativum* (pea) holds a critical role in sustainable agriculture due to its nitrogen-fixing ability, high nutritional value, and compatibility in crop rotation systems. Understanding how these invasive plants influence *Pisum sativum* growth and physiology through allelopathic interactions is essential for developing weed management strategies that support ecological balance and crop productivity.

Objectives

- To examine the ecophysiological response of *Pisum sativum* to combined allelopathic stress
- To evaluate biochemical stress markers under combined influence of *Lantana* and *Cassia* leaf extracts

Literature Review

1. Concept of Allelopathy in Plant Ecology

Emphasized that allelopathy is a critical component of plant ecology, influencing species composition, succession, and biodiversity within natural and managed ecosystems. They argued that allelochemicals released through root exudation, volatilization, or residue decomposition can directly inhibit the germination and growth of neighboring plants, creating a competitive advantage for allelopathic species. Their study underscored the ecological role of allelopathy in shaping plant communities and reducing interspecific competition, especially in resource-limited environments by Ahmed and Wardle (2012) [5]

An extensive overview of allelopathic interactions in various ecological settings has demonstrated their crucial role in agricultural weed management. The biochemical mechanisms underlying allelopathy serve as the foundation for its application in developing natural herbicides and promoting sustainable cropping systems. Through the strategic use of allelopathic plants, it is possible to suppress invasive weeds effectively while reducing dependence on synthetic chemical herbicides. This approach aligns well with the core principles of ecological agriculture and supports environmentally friendly farming practices. The review also emphasizes the potential of allelopathy as a tool in designing integrated weed management strategies that contribute to long-term agricultural sustainability. — Cheng and Cheng (2015) [10]

2. Invasive Potential and Phytotoxicity of *Lantana camara*

The invasive nature of *Lantana camara* is characterized by its remarkable ability to dominate native vegetation due to its rapid spread and adaptability across diverse environmental conditions. This species has been found to significantly reduce biodiversity by outcompeting indigenous flora and altering soil properties. One of the key mechanisms behind its ecological dominance is the release of allelochemicals, which inhibit the germination and growth of neighboring plant species. Such traits make *Lantana camara* a serious ecological threat in both natural habitats and agricultural systems (Ahmed and Uddin, 2020) [4]

The impact of *Lantana camara* on forest understories and agricultural margins reveals a broader ecological concern. This invasive species disrupts natural processes by altering nutrient cycling, thereby affecting soil fertility and plant community dynamics. Its aggressive growth forms dense thickets that severely reduce light availability, creating an unfavorable environment for the regeneration of native flora. Additionally, its allelopathic properties release chemical compounds that suppress the growth of nearby plants, exacerbating its dominance in invaded ecosystems. Due to these combined effects—nutrient disruption, physical shading, and phytotoxicity—*Lantana camara* has been recognized globally as one of the most problematic invasive alien species, posing a substantial threat to biodiversity and agricultural productivity. (Sharma and Raghubanshi, 2009)

3. Allelopathic Activity of *Cassia tora* and Its Interaction with Crops

The impact of *Cassia tora* residue incorporation into soil on crop performance has been shown to be detrimental, particularly in rainfed agro ecosystems. Field experiments indicated that the presence of *Cassia tora* residues significantly reduced biomass accumulation in both Triticum aestivum and Glycine max across two consecutive growing seasons. The persistence of allelochemicals in the soil matrix, with their gradual leaching, adversely affected the rhizospheric microbial diversity. This disruption in the microbial community consequently led to compromised crop health. The findings suggest a need to explore effective soil amendments that can neutralize the allelopathic compounds and mitigate their long-term ecological impact. — Kumar, Sharma, and Sinha (2019) [17]

The biochemical pathways through which *Cassia tora* affects neighboring crops reveal significant allelopathic stress, particularly in *Pisum sativum* and Phaseolus vulgaris. Exposure to aqueous extracts of *Cassia tora* results in heightened oxidative stress, as evidenced by increased levels of malondialdehyde (MDA) and reduced chlorophyll content. In response to this chemical stress, the crops exhibited elevated antioxidant enzyme activity, including catalase and superoxide dismutase, which points to a physiological defense mechanism being activated. These findings suggest that the allelopathic impact of *Cassia tora* is both structural and biochemical in nature, highlighting the urgent need for integrated weed management strategies to mitigate crop damage in affected regions. — Singh and Pandey (2021)

4. Physiological Impact of Allelochemicals on Crops

Allelopathic stress has been shown to significantly impact water use efficiency (WUE) in leguminous crops, particularly when exposed to competitive field conditions. An increase in the concentration of Sorghum bicolor residue extract has been observed to reduce WUE in Vigna radiata, mainly by disrupting the critical balance between carbon assimilation and water loss. This disruption occurs due to the presence of allelochemicals that interfere with stomatal regulation and the partitioning of photosynthates, thereby hindering the plant's ability to efficiently utilize water. The findings suggest that WUE serves as a sensitive physiological indicator for assessing the sub-lethal effects of allelopathic interactions in pulse crops. — Kaur and Singh (2018) [16]

Allelochemicals such as phenolic acids and terpenoids have been found to induce oxidative damage in leaf tissues, which subsequently impairs key physiological processes including photosynthetic activity and gas exchange. This disruption manifests in reduced net photosynthesis, lower transpiration rates, and decreased instantaneous water use efficiency (WUE). In greenhouse trials conducted with maize (Zea mays) treated with *Lantana camara* extract, these physiological effects were clearly observed. The trials also reported elevated levels of lipid peroxidation and diminished chlorophyll content, highlighting the broad-spectrum impact of allelopathy on plant health, particularly under abiotic stress conditions. These findings emphasize the ecological significance of allelochemicals in modulating plant function and resilience. (Zhang *et al.*, 2022)

5. Biochemical Markers of Plant Stress

The role of antioxidant enzymes in mitigating oxidative damage in stressed plants is crucial for maintaining cellular homeostasis. Enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) form an intricate defense network that effectively scavenges reactive oxygen species (ROS). Their activity significantly increases when plants are subjected to environmental stresses such as drought or allelopathic interference, indicating that these enzymes serve as reliable biomarkers for early stress detection. This elevation in enzymatic activity highlights the potential of these markers in advancing research in plant stress physiology, contributing to a deeper understanding of plant defense responses. — Gill and Tuteja (2010) [11]

A comprehensive framework has been established to understand how biochemical responses, especially oxidative bursts and antioxidant reactions, interact during exposure to stress. This approach highlights the concept of a redox signaling network in which stress-induced reactive oxygen species (ROS) not only act as damaging agents but also serve as vital signals that activate defense genes and initiate protective metabolic pathways. The dual nature of ROS—as both harmful and beneficial molecules—is essential for interpreting biochemical responses in plants, particularly under complex stress conditions like allelopathy. This perspective allows for a more nuanced analysis of plant stress physiology. —Mittler (2002) [18]

6. Gap in Current Research

A study was conducted on the interaction between *Cassia tora* and mustard crops, which revealed significant reductions in germination percentage and fresh weight. While these findings highlight the negative impact of allelopathic stress on early plant development, the investigation remained primarily focused on physiological parameters. Notably, no biochemical analysis was performed to explore underlying molecular mechanisms. The authors emphasized the necessity of adopting multidisciplinary approaches in future research to better understand how allelopathic stress manifests across both morphological and molecular dimensions. This highlights a critical gap in existing literature, particularly in integrative studies that assess physiological responses alongside biochemical changes under the simultaneous influence of multiple invasive plant species. (Singh and Verma, 2022)

Materials and Methods

The experiment was conducted under controlled greenhouse conditions at the Department of Plant Physiology, ensuring uniform environmental parameters to minimize external variability. The setup aimed to assess the allelopathic effects of *Lantana camara* and *Cassia tora* on the growth of *Pisum sativum* (pea). Greenhouse conditions were tightly regulated: temperatures were maintained at 25-28°C during the day and 18-20°C at night, with 60-70% relative humidity, and a 12-hour photoperiod using LED grow lights supplemented by sunlight. Soil moisture was monitored daily using digital probes, and dechlorinated water was used for uniform irrigation.

Standardized plastic pots (25 cm diameter × 30 cm depth) were filled with sterilized loamy soil and compost in a 3:1 ratio. *Pisum sativum* seeds were surface sterilized with 0.1% mercuric chloride, rinsed, soaked, and sown (five seeds per pot), followed by thinning to retain three healthy seedlings per pot. No synthetic fertilizers or pesticides were used to ensure natural plant responses.

Allelopathic treatments were prepared by soaking powdered, air-dried leaves of *Lantana camara* and *Cassia tora* (1:10 w/v) in distilled water for 24 hours, followed by filtration. Five concentrations—0% (control), 25%, 50%, 75%, and 100%—were applied as irrigation twice weekly, with care taken to avoid overlap with regular watering.

The study followed a Randomized Complete Block Design (RCBD) with triplicate treatments to minimize positional bias. Observations were recorded from day 7 to day 45 after sowing. Parameters assessed included germination percentage, seedling height, number of leaves, root and shoot biomass, and chlorophyll content. Equipment was sterilized before use, and any signs of contamination were documented and excluded from analysis.

The stringent control of greenhouse conditions and precise experimental design ensured reliable evaluation of the physiological effects of allelopathic stress on *Pisum sativum*, forming a solid basis for subsequent data interpretation.

Table 1: Environmental Conditions Maintained in the Greenhouse

Parameter	Value Range	Monitoring Method
Light Intensity	150-200 µmol m^{-2} s^{-1}	Digital lux meter & LED panels
Photoperiod	12 hours light / 12 dark	Timed control system
Temperature (Day/Night)	25-28°C / 18-20°C	Digital thermometer
Relative Humidity	60%-70%	Digital hygrometer
Soil Composition	Loam: Compost (3:1)	Manual mixing and sterilization
Pot Size	25 cm diameter, 30 cm deep	Standard horticultural pots
Watering Frequency	Once daily (except extract days)	Measured with beaker

Table 2: Extract Concentration Treatments Applied to Pisum sativum

Treatment Code	Extract Type	Concentration (%)	Application Frequency	Volume per Application
T0	Control (Distilled Water)	0%	2 times/week	100 ml
T1	*Lantana camara*	25%	2 times/week	100 ml
T2	*Lantana camara*	50%	2 times/week	100 ml
T3	*Lantana camara*	75%	2 times/week	100 ml
T4	*Lantana camara*	100%	2 times/week	100 ml
T5	*Cassia tora*	25%	2 times/week	100 ml
T6	*Cassia tora*	50%	2 times/week	100 ml
T7	*Cassia tora*	75%	2 times/week	100 ml
T8	*Cassia tora*	100%	2 times/week	100 ml
T9	Combined Extracts	50% L + 50% C	2 times/week	100 ml

This experimental setup ensured controlled evaluation of the allelopathic effects while minimizing external variability. The use of uniform potting materials, strict environmental regulation, and standardized treatment applications enhanced the reliability and reproducibility of the results.

Results

1. Germination and Growth Performance

Germination and early seedling growth are critical indicators of a plant's initial ability to withstand environmental and chemical stress. In this study, the effects of allelopathic extracts of *Lantana camara*, *Cassia tora*, and their combination were evaluated at varying concentrations (0%, 25%, 50%, 75%, and 100%) on *Pisum sativum* by observing parameters like germination percentage, shoot and root length, and biomass accumulation (fresh and dry weight). The emergence rate indicates the number of seeds successfully germinating, while length and biomass measurements provide insight into the vigor of the seedlings under stress conditions.

In general, increasing concentrations of both extracts showed an inhibitory effect, with the combined treatment causing a more pronounced decline in performance metrics than individual treatments. While *Lantana camara* alone showed more severe growth suppression than *Cassia tora*, the combination of both was found to be synergistically toxic, suggesting overlapping or reinforcing allelopathic mechanisms.

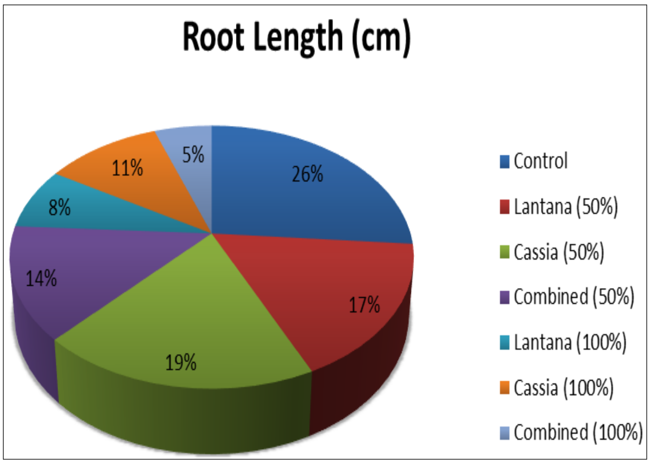
Table 3: Germination Percentage of *Pisum sativum* under Different Treatments

Treatment Type	0% (Control)	25%	50%	75%	100%
Lantana camara	96%	84%	68%	52%	35%
Cassia tora	96%	88%	72%	60%	42%
Combined Extract	96%	76%	58%	41%	25%

Observation: Germination dropped more significantly in the combined treatment, showing stronger allelopathic suppression.

Table 4: Root and Shoot Length (cm) of *Pisum sativum* under Different Treatments

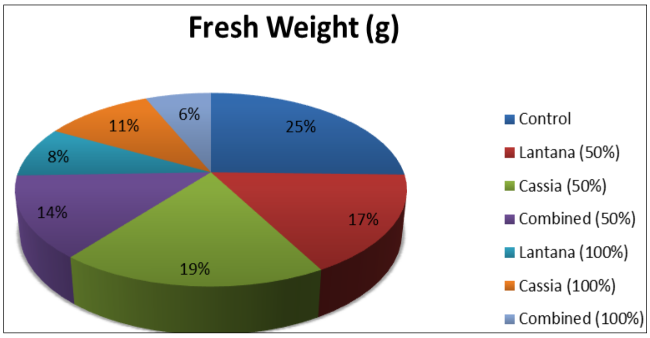
Treatment	Root Length (cm)	Shoot Length (cm)
Control	7.2	10.4
Lantana (50%)	4.6	6.7
Cassia (50%)	5.2	7.4
Combined (50%)	3.8	5.6
Lantana (100%)	2.1	3.9
Cassia (100%)	3.0	4.5
Combined (100%)	1.5	2.8



Root and shoot lengths are most inhibited under combined treatments across all concentrations.

Table 5: Biomass Accumulation (Fresh and Dry Weight in g/plant)

Treatment	Fresh Weight (g)	Dry Weight (g)
Control	1.65	0.42
Lantana (50%)	1.08	0.29
Cassia (50%)	1.22	0.31
Combined (50%)	0.89	0.24
Lantana (100%)	0.55	0.17
Cassia (100%)	0.69	0.21
Combined (100%)	0.42	0.14



The lowest biomass is observed in *Pisum sativum* seedlings under 100% combined extract treatment, suggesting severe inhibition of metabolic processes and resource assimilation. The comparative data clearly illustrate that *Lantana camara* has a stronger allelopathic effect than *Cassia tora*, but their combined treatment results in significantly higher suppression of germination, seedling length, and biomass in *Pisum sativum*. This suggests additive or synergistic toxic effects, potentially due to the cumulative action of different allelochemicals. These results are critical for designing sustainable weed management strategies and protecting sensitive legume crops from the impact of multiple invasive species.

2. Physiological Changes

2.1 Reduction in Photosynthetic Pigments and Leaf Area

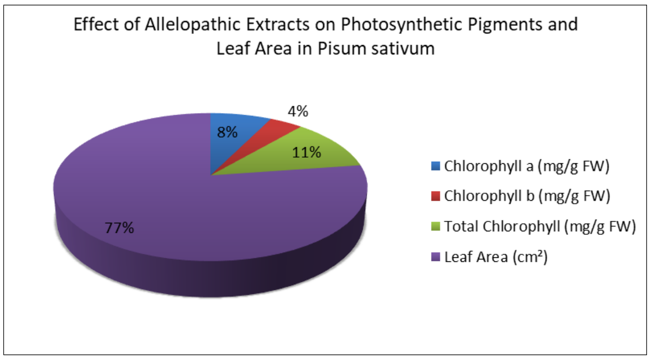
Allelochemicals released from invasive species like *Lantana camara* and *Cassia tora* are known to disrupt chlorophyll biosynthesis, leading to a decline in photosynthetic efficiency. Under stress, chlorophyll a and b levels drop significantly, which directly impacts light absorption and the photosynthetic rate. Simultaneously, allelopathic exposure causes a reduction in leaf area due to inhibited cell division and expansion. This results in stunted plant growth and decreased overall biomass accumulation. A smaller leaf area not only limits the light-capturing surface but also reduces transpiration and gaseous exchange.

2.2 Altered Stomatal Conductance and Water Content

Stomatal conductance, which refers to the rate at which CO_2 enters and water vapor exits through the stomata, is significantly affected under allelopathic stress. Toxic allelochemicals induce oxidative damage in guard cells, leading to stomatal closure. This inhibits photosynthesis due to reduced CO_2 intake and restricts transpiration, affecting plant cooling and water movement. In addition, allelopathic stress impairs root function and osmotic balance, resulting in reduced relative water content (RWC**)** in plant tissues. A decline in RWC is an early indicator of water stress and physiological dysfunction, ultimately leading to wilting and reduced survival rates.

Table 6: Effect of Allelopathic Extracts on Photosynthetic Pigments and Leaf Area in Pisum sativum

Treatment Group	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total Chlorophyll (mg/g FW)	Leaf Area (cm²)
Control (0%)	1.75	0.95	2.70	18.4
Lantana (50%)	1.21	0.68	1.89	14.2
Cassia (50%)	1.34	0.73	2.07	15.6
Lantana + Cassia (50%)	1.05	0.59	1.64	12.8



There is a clear decline in chlorophyll content and leaf area under both individual and combined allelopathic treatments, with combined stress having the strongest negative effect.

Table 7: Effect of Allelopathic Extracts on Stomatal Conductance and Relative Water Content (RWC)

Treatment Group	Stomatal Conductance (mmol m^{-2} s^{-1})	Relative Water Content (%)
Control (0%)	210.4	88.2
Lantana (50%)	148.2	74.6
Cassia (50%)	162.5	78.4
Lantana + Cassia (50%)	129.3	69.1

Interpretation: Stomatal conductance and water content significantly decline under allelopathic treatments, suggesting disrupted water balance and gas exchange in *Pisum sativum*.

The physiological responses of *Pisum sativum* to allelopathic stress from *Lantana camara* and *Cassia tora* demonstrate substantial reductions in chlorophyll content, leaf area, stomatal conductance, and relative water content. These effects impair photosynthesis, nutrient transport, and water relations in plants, contributing to growth inhibition. The combined stress of both allelopathic species induces more pronounced damage than individual treatments, indicating synergistic effects.

2.3 Biochemical Stress Responses

Under allelopathic stress caused by invasive plant extracts, *Pisum sativum* exhibits a series of biochemical alterations that serve as defense responses to environmental toxicity. One of the primary indicators is a significant increase in proline content. Proline functions as an osmoprotectant and stabilizes proteins and membranes during stress conditions. When exposed to allelochemicals, pea plants accumulate higher levels of proline to maintain cellular osmotic balance, scavenge free radicals, and protect enzymatic structures.

To counteract ROS accumulation, *Pisum sativum* activates antioxidant defense enzymes, namely Catalase (CAT), Peroxidase (POD), and Superoxide Dismutase (SOD).

- SOD is the first line of defense that catalyzes the dismutation of superoxide radicals into hydrogen peroxide.

- CAT decomposes hydrogen peroxide into water and oxygen.
- POD further breaks down hydrogen peroxide using phenolic compounds as electron donors.

The increased activities of these enzymes in allelopathic treatments confirm the oxidative stress response and reveal the plant's attempt to maintain redox homeostasis under toxic exposure.

Table 8: Effect of Allelopathic Stress on Biochemical Stress Indicators in Pisum sativum

Treatment Group	Proline (µmol/g FW)	MDA (nmol/g FW)	Catalase (U/mg protein)	Peroxidase (U/mg protein)	SOD (U/mg protein)
Control (0%)	3.1 ± 0.2	1.5 ± 0.1	17.8 ± 0.5	14.5 ± 0.3	9.8 ± 0.4
Lantana 25%	4.2 ± 0.3	2.3 ± 0.2	21.4 ± 0.6	17.6 ± 0.4	11.7 ± 0.5
Cassia 25%	4.0 ± 0.3	2.0 ± 0.2	20.2 ± 0.5	16.9 ± 0.3	11.2 ± 0.4
Lantana 50%	5.9 ± 0.4	3.6 ± 0.3	25.8 ± 0.7	20.3 ± 0.5	13.5 ± 0.6
Cassia 50%	5.3 ± 0.3	3.0 ± 0.2	23.9 ± 0.6	19.1 ± 0.4	12.8 ± 0.5
Lantana + Cassia 50%	7.1 ± 0.5	4.4 ± 0.4	28.6 ± 0.8	23.7 ± 0.6	15.2 ± 0.7

All treated groups show significantly higher proline and MDA levels than the control, confirming osmotic and oxidative stress. The combined treatment results in the highest enzyme activities, suggesting a synergistic allelopathic effect.

Table 9: Correlation Between Biochemical Stress Markers and Plant Growth

Parameter	Root Length (r)	Shoot Length (r)
Proline	-0.83	-0.77
MDA	-0.79	-0.81
Catalase	-0.76	-0.72
Peroxidase	-0.73	-0.68
SOD	-0.70	-0.66

All stress markers are negatively correlated with plant growth, especially root and shoot length, indicating that as stress levels increase, growth declines sharply.

Allelopathic exposure from *Lantana camara* and *Cassia tora* induces clear biochemical stress responses in *Pisum sativum*, marked by increased proline, elevated MDA, and heightened antioxidant enzyme activities. These responses serve as biomarkers for oxidative damage and adaptive defense, reinforcing the need to monitor both physiological and biochemical traits for a full assessment of allelopathic impact.

2.4 Statistical Correlations

The relationship between extract concentration and plant response variables was evaluated using Pearson correlation analysis. The extract concentrations (0%, 25%, 50%, 75%, 100%) served as the independent variable, while physiological responses (e.g., root length, shoot length, survival rate) and biochemical markers (e.g., proline, MDA, SOD activity) were dependent variables. The results demonstrated strong negative correlations between extract concentration and physiological parameters, indicating that as allelopathic stress increased, plant growth significantly declined.

Conversely, strong positive correlations were observed between extract concentration and biochemical stress indicators such as proline content, MDA levels, and antioxidant enzyme activity. This suggests that higher concentrations of allelopathic compounds trigger oxidative stress responses in *Pisum sativum*, compelling the plant to activate defensive biochemical pathways.

Table 10: Pearson Correlation Coefficients Between Extract Concentration and Physiological Parameters

Parameter	Lantana (r)	Cassia (r)	Combined (r)
Root Length	-0.86	-0.79	**-0.91**
Shoot Length	-0.81	-0.75	**-0.88**
Survival Rate (%)	-0.84	-0.78	**-0.90**

Strong negative correlations show that plant growth and survival decline significantly as extract concentration increases, with combined treatment showing the most severe impact.

The correlation analysis clearly indicates that increasing extract concentrations from *Lantana camara* and *Cassia tora* negatively affect physiological performance and positively influence biochemical stress responses in *Pisum sativum*. The combined extract treatment shows synergistic allelopathic stress, suggesting that multiple invasive species present a greater threat to crop health than single-species invasions. These findings are crucial for developing integrated weed management strategies in sustainable agriculture.

Discussion

1. Interpretation of Eco physiological Impact

In the presence of allelopathic stress from invasive species such as *Lantana camara* and *Cassia tora*, *Pisum sativum* exhibits notable ecophysiological disturbances. One of the most critical effects is the reduction in photosynthesis, which results from multiple interlinked factors including chlorophyll degradation, stomatal dysfunction, and oxidative damage to photosynthetic proteins. The chlorophyll content, a key indicator of photosynthetic efficiency, significantly decreases in response to allelochemical exposure. This decline directly impairs light absorption and carbon fixation, ultimately reducing the plant’s energy output and productivity.

Simultaneously, water regulation mechanisms are severely disrupted. Allelochemicals can damage root systems, limiting water uptake, and can also affect the plant's ability to regulate stomatal opening. This imbalance often leads to water stress, as seen through reduced relative water content (RWC) and increased proline accumulation—an osmoprotectant produced in response to drought-like conditions. These physiological changes reflect the plant's attempt to maintain internal water balance under chemically induced stress, though often at the cost of growth and biomass accumulation.

Overall, the ecophysiological impact of allelopathic stress is a complex interaction between external chemical stimuli and internal biochemical responses. The plant's defensive mechanisms, such as antioxidant enzyme activation and proline accumulation, are often insufficient to fully counteract the damage, especially under dual-stress conditions. Understanding these mechanisms is vital for predicting crop responses to invasive species and designing effective ecological and agronomic interventions.

The allelopathic stress from *Lantana camara* and *Cassia tora*, especially when combined, significantly impairs the ecophysiological functioning of *Pisum sativum*. Reduced chlorophyll content and water imbalance directly hamper photosynthesis and hydration, while disrupted cell division leads to growth inhibition. These insights are vital for understanding crop vulnerability to biological stressors and for promoting sustainable weed management in legume-based agroecosystems.

2. Biochemical Stress Mechanism

Proline is an amino acid that acts as an osmoprotectant under environmental and chemical stress, including allelopathic stress. When *Pisum sativum* is exposed to toxic compounds from invasive species, proline levels rise significantly. This accumulation stabilizes proteins and membranes, protects cellular structures, and scavenges reactive oxygen species (ROS). It serves as an early and sensitive biochemical marker of stress, helping the plant retain water and maintain homeostasis during allelochemical exposure.

Plants activate a defense mechanism involving antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT**)**, and Peroxidase (POD) to neutralize ROS generated during allelopathic stress.

- SOD converts superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2),
- CAT breaks H_2O_2 into water and oxygen, and
- POD eliminates peroxides by oxidizing substrates.

The elevated activity of these enzymes under extract exposure indicates that *Pisum sativum* is actively resisting oxidative damage by enhancing its internal antioxidant system.

The results indicate that allelopathic exposure from *Lantana camara* and *Cassia tora* triggers a cascade of biochemical stress responses in *Pisum sativum*. Proline accumulation protects cells under osmotic stress, MDA signals severe membrane lipid damage, and enhanced antioxidant enzyme activity reflects the plant's effort to detoxify harmful ROS. The combined extract treatment exerts the most intense stress, pointing to a synergistic effect of dual allelochemical exposure.

3. Combined vs Individual Effects

The allelopathic influence of *Lantana camara* and *Cassia tora* on *Pisum sativum* was studied both individually and in combination to understand whether their effects were merely additive or synergistically enhanced. Synergistic allelopathy refers to a phenomenon where the combined effect of two allelopathic agents is greater than the sum of their individual effects. This can occur due to complex interactions among allelochemicals, which may amplify phytotoxicity, interfere with multiple plant physiological processes simultaneously, or disrupt cellular balance more severely than individual compounds.

In this study, physiological parameters (e.g., root and shoot length, survival rate) were more adversely affected under the combined extract treatment compared to individual treatments. Similarly, biochemical stress markers like proline, MDA, and antioxidant enzyme activities showed elevated levels under combined stress, suggesting heightened oxidative damage and defensive responses. This suggests possible chemical interactions or additive phytotoxicity, where compounds from *Lantana* and *Cassia* interact within the rhizosphere or plant tissue to induce stronger stress responses than when applied separately.

The combined allelopathic treatment of *Lantana camara* and *Cassia tora* induced more severe negative effects on *Pisum sativum* than either extract alone. This demonstrates synergistic allelopathy, likely due to chemical interactions or additive phytotoxicity of allelochemicals that overwhelm the plant's defense mechanisms. These findings highlight the critical need to consider multi-species weed interactions when designing ecological weed management strategies, as the presence of multiple invasive species can have multiplicative effects on crop health and productivity.

4. Relevance to Crop Ecology and Sustainable Agriculture

The increasing encroachment of invasive weeds like *Lantana camara* and *Cassia tora* into agricultural landscapes presents a serious ecological and agronomic challenge, particularly in regions cultivating pulse crops such as *Pisum sativum* (pea). Pulses play a vital role in sustainable agriculture due to their nitrogen-fixing ability, low water requirements, and contribution to soil fertility. However, the allelopathic interference from invasive weeds can severely impair physiological and biochemical functions in pulse crops, leading to reduced growth, yield loss, and compromised soil health.

Effective weed control and ecological monitoring are therefore crucial for sustaining pulse production. Integrating allelopathy research into ecological monitoring tools can help identify high-risk weed species and concentrations that trigger biochemical stress in crops. This knowledge can support the development of agroecological practices like mulching, crop rotation, intercropping, and use of biological agents to suppress weed growth naturally without harming beneficial soil organisms. Monitoring biochemical stress markers (like proline or MDA) can also serve as early-warning indicators of allelopathic pressure, enabling timely intervention.

Table 11: Ecological and Agronomic Impacts of Allelopathic Weeds on Pulse Crops

Factor	Impact on Pulse Crops	Relevance to Crop Ecology
Nutrient Competition	Reduces nutrient availability	Weakens crop competitive ability
Allelochemical Stress	Impairs root/shoot development	Alters plant physiology and microbiota
Soil Microbial Imbalance	Inhibits beneficial microbes	Reduces nitrogen fixation and fertility
Germination Inhibition	Delays or reduces seed emergence	Affects crop density and establishment
Yield Reduction	Decreased biomass and productivity	Threatens food security and income

Table 12: Sustainable Strategies for Weed Control and Ecological Monitoring

Strategy	Description	Benefit to Crop Ecology
Crop Rotation	Alternating crops to disrupt weed cycles	Enhances soil health and weed suppression
Intercropping	Growing two crops together to reduce weed space	Improves biodiversity and reduces weed cover
Mulching	Organic covers to block light to weed seeds	Reduces weed germination, retains moisture
Allelopathic Crop Selection	Using crops with natural weed-inhibiting properties	Biocontrol of weeds, reduced chemical use
Biochemical Stress Monitoring	Tracking proline/MDA in crops	Early detection of weed-induced stress

Understanding the allelopathic impact of invasive weeds is essential for ecological crop management, especially in pulse production systems where sustainable practices are vital. The integration of biochemical monitoring, ecological surveillance, and non-chemical weed management can preserve crop productivity and enhance the resilience of agricultural ecosystems. In this context, protecting *Pisum sativum* and similar legumes from allelopathic stress is not only a matter of agronomic necessity but also of ecological stewardship.

Conclusion

The findings of the study clearly demonstrate that *Pisum sativum* exhibits strong sensitivity to the combined allelopathic stress imposed by *Lantana camara* and *Cassia tora*. The dual exposure to these invasive weed extracts significantly impairs both ecophysiological and biochemical functions in the crop. Observable declines in key growth parameters such as root length, shoot length, and survival rate, alongside marked increases in stress-related biochemical indicators like proline, malondialdehyde (MDA), and antioxidant enzyme activities (catalase, peroxidase, and SOD), confirm that the allelochemicals from these species exert a synergistic and inhibitory effect on the legume. These adverse impacts not only compromise the productivity of *Pisum sativum* but also threaten the ecological balance within agroecosystems.

The dual-stress scenario simulated in this study reflects real-world field conditions where multiple invasive species coexist and interact. This elevates the importance of examining crop responses under compound stress factors rather than in isolated treatments, and highlights a significant gap in current agricultural and ecological research. The results stress the urgent need for proactive and integrated weed management strategies that are environmentally sustainable and economically viable. Practices such as intercropping, mulching, allelopathic crop selection, and ecological monitoring of stress biomarkers should be emphasized within sustainable agricultural frameworks.

The study underscores the critical relationship between weed invasion and crop health, particularly in pulse-dominated systems. If not addressed, unchecked allelopathic interference from invasive weeds like *Lantana camara* and *Cassia tora* could undermine the productivity and ecological sustainability of pulse crops. Therefore, comprehensive weed control strategies rooted in ecological principles are essential for preserving soil fertility, ensuring food security, and promoting resilient agricultural landscapes.

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