

Allelochemical Fingerprinting of Invasive Weeds and Their Biochemical Interference with Seed Germination and Enzymatic Metabolism of Crops

Shalini Priya Kapoor ^{1*}, Aditya Varun Deshmukh ²

¹⁻² Institute of Biomaterials and Cancer Nanotherapeutics, University of Delhi, India

*Corresponding author: **Shalini Priya Kapoor**

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ABSTRACT

Background: Background: Invasive plants have spread to different ecosystems around the planet to an extent that the role of allelopathy is one of the dominant reasons for their success. Allelopathy refers to the ability of plants to secrete certain chemicals (allelochemicals) that affect the ability of other plants to germinate and metabolise crop species. The development of gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and similar methods has made it possible to formulate chemical profiles of different invasive plant species and ecosystems, but the application of chemical fingerprinting in understanding the ways toxic chemicals influence plants remains limited.

Objective: This review summarises the literature sources published between 2015 and 2025 (important studies published before 2015 have been included where appropriate) discussing the identification of allelochemicals of eco-economically important invasive plant species and their biochemical influence on germination and metabolism of crops.

Sources of literature: A meticulous combination search of Scopus, Web of Science, PubMed and Google Scholar resulted in a total of 116 studies included in qualitative thematic analysis after screening based on PRISMA.

Main findings: Phenolic acids (including ferulic, p-coumaric, gallic and vanillic), sesquiterpene lactones (parthenin), quinones (sorgoleone) and flavonoids repeatedly show up in large quantities among alien species such as *Parthenium hysterophorus*, *Mikania micrantha*, *Chromolaena odorata*, *Ageratum conyzoides* and *Sorghum halepense*. These various components contribute to inhibition of α -amylase and protease activities during soaking which lead to ROS accumulation, superoxide dismutase, catalase and peroxidase disturbance, as well as lipid peroxidation resulting in diminished germination, radicle growth and growth score in crops.

Research gaps: Reviewed evidence is dominated by laboratory bioassays using aqueous or organic extracts at arbitrary concentrations, with limited field-scale validation, inconsistent dose standardisation, sparse integration of multi-omics platforms, and negligible attention to soil-microbiome-mediated transformation or climate-change interactions.

Conclusion: Allelochemical fingerprinting offers a mechanistically grounded route toward both improved invasive weed management and the rational design of selective bioherbicides, but the field requires methodological standardisation, field validation and multi-omics integration before this promise can be translated into applied crop protection.

Keywords: allelopathy; invasive weeds; allelochemical fingerprinting; seed germination; antioxidant enzymes; GC-MS metabolomics; crop physiology; bioherbicide

1. Introduction

1.1. Background and Global Scenario

Weeds invading agriculture and ecosystems have a huge, long-lasting negative economic impact on agriculture and natural ecosystems through a process called biological invasion. An Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) report from 2023 indicated that the documented annual global cost of invasive alien species is over \$423 billion ^[33]. This is a near quadrupling each decade since 1970, and agriculture has made a disproportionate contribution to the total reported loss of agricultural production ^[33]. Over six decades, estimates ranging from several hundred billion to over one trillion dollars have reported related to economic analyses of invasive species. Weeds have contributed greatly through suppressing yields, contaminating harvested products, and the cost of managing weeds. There is growing evidence that indicate allelopathy—the chemical inhibition of neighbouring plants—has allowed invasive weeds to become dominant and could be used as a mechanism for establishing dense, long-lived, low-diversity patches of invasives in croplands.

Rice ^[39] originally defined allelopathy as the process whereby plants exert beneficial or detrimental influences on other plants through the release of chemicals into their environment. This definition has since been significantly broadened through the study of the biochemical mechanisms involved with these types of interactions over the past several decades ^[40]. Major global invasive weeds such as *Chromolaena odorata*, *Parthenium hysterophorus*, *Mikania micrantha*, *Ageratum conyzoides*, *Lantana camara*, and *Sorghum halepense* (commonly referred to as Johnson grass) release highly complex combinations of chemical allelochemicals including phenolics, terpenoids, alkaloids, and quinones from their root, leaf and flower tissues as well as from their decomposing plant residues ^[4, 15]. However, these allelochemicals do not uniformly affect all plants, but rather target specific physiological and biochemical

check-points in the seed germination process of target (susceptible) crop seeds, ranging from seed imbibition and the mobilization of stored energy to the functioning of antioxidant defence systems during the germination of plant tissues to protect them from damage by reactive oxygen species.

1.2. Importance of the Subject

During the crop life cycle, seed germination is considered the most vulnerable time period, with even small amounts of allelochemicals affecting this stage dramatically evidenced by the reduction of field emergence, establishment of stands, and ultimately yield. Enzymatic metabolism during the germination process via enzymatic hydrolysis (i.e., α -amylase, protease) of stored reserves as well as regulation via antioxidant enzymes (i.e., SOD, CAT, POD) of reactive oxygen species (ROS) provides a sensitive and mechanistically informative endpoint for characterising allelochemical toxicity [23, 24]. Over the last decade, the chemical "fingerprinting" of allelochemicals derived from weeds using GC-MS/LC-MS and other metabolomic-based analytical processes has progressed significantly, allowing a transition from non-specific bioassays using aqueous extracts towards the identification of specific bioactive components and where relevant molecular targets [18, 21]. The convergence of plant physiology and analytical chemistry thus has direct relevance for two applied areas: (i) the development of early warning diagnostic tools to predict allelopathic risk from new naturalised weed species, and (ii) the rational design of selective plant based bioherbicides to provide alternatives to synthetic agrochemicals.

1.3. Need for the Review

The growing body of primary literature on allelochemical fingerprints and their subsequent biochemical effects is currently split among weed science, analytical chemistry, ecology, and plant physiology publishing outlets; with large variation in extraction techniques, published concentrations, bioassay-species, and end-points. There has not been a recent synthesis that has systematically mapped the association between classes of allelochemicals and their ability to disrupt

different enzymes across the range of taxonomically diverse invasive weeds; nor has the methodological quality of the dose-response studies or fingerprinting studies been thoroughly examined. The fragmentation of this literature limits the translational use of what is otherwise an extensive body of literature, thereby establishing the rationale for this review paper.

1.4. Objectives and Scope of the Review

The purpose of this review is: (i) to provide a synthesis of the major analytical methods used for allelochemical fingerprinting of invasive species; (ii) to critically evaluate and compare the effects of allelochemicals on crop seed germination, including both hydrolytic and antioxidant enzyme systems; (iii) to identify areas of agreement, disagreement and methodological limitations in the literature examined; and (iv) to delineate research gaps and emerging technological trends. As such, the review is limited to terrestrial invasive weed species with allelopathic effects on agronomically important crops, using literature published primarily between 2015-2025 with additional references to landmark studies that established basic mechanistic principles.

2. Methodology of Literature Review

2.1. Literature Search Strategy

A systematic search of the literature has been carried out across four different databases (Scopus, Web of Science, PubMed, and Google Scholar), searching for articles published between January 2015 and March 2025, but with the inclusion of older classical studies that provided significant aspects of mechanism. The methodology involved using both controlled vocabulary and free-text terms as part of Boolean search strings, building the Boolean logic from the following terms: (invasive weed* OR alien plant*) AND (allelochemical* OR allelopathy) AND (fingerprint* OR GC-MS OR metabolomic*) AND (seed germination OR enzyme* OR antioxidant OR amylase). A summary of the details of the search strategy and syntax used to search each database can be found in Table 1 (attached).

Table 1: Literature Search Strategy Applied Across Databases

Database	Search string / field	Records retrieved	Search period
Scopus	TITLE-ABS-KEY("invasive weed" AND allelochemical AND (germination OR enzyme))	612	2015–2025
Web of Science	TS=(allelopathy AND "invasive plant*" AND ("seed germination" OR "antioxidant enzyme"))	498	2015–2025
PubMed	(allelochemical[Title/Abstract]) AND (germination OR enzyme activity)	214	2015–2025
Google Scholar	"allelochemical fingerprinting" invasive weed crop germination enzyme	387	2015–2025

2.2. Inclusion and Exclusion Criteria: Studies were screened against pre-defined inclusion and exclusion criteria

(Table 2) applied sequentially at the title/abstract and full-text stages.

Table 2: Inclusion and Exclusion Criteria for Study Selection

Criteria type	Inclusion	Exclusion
Publication type	Peer-reviewed original research and reviews in indexed journals	Conference abstracts, non-peer-reviewed reports, theses without journal output
Language	English-language full text available	Non-English publications without English translation
Topical focus	Documented allelochemical identification AND at least one germination or enzymatic endpoint in a crop or model plant	Studies on animal, microbial or purely ecological (non-biochemical) allelopathy endpoints
Publication period	2015–2025 (landmark mechanistic studies pre-2015 retained where directly relevant)	Superseded/duplicate datasets; near-identical re-publications
Data quality	Clearly reported extraction method, concentration/dose, and statistical analysis	Studies lacking methodological detail sufficient for quality appraisal

2.3. Study Selection Process (PRISMA-Style)

A PRISMA flow diagram adapted from Page *et al.* [38] was used for reviewing the studies. In total 1711 records were identified and 1213 records remained after the removal of duplicates. Title/abstract screening resulted in exclusions of 861 records as out of scope (i.e., not related to plant-based allelochemical/crop interactions), leaving a total of 352 articles to assess for eligibility through a full text review. A further 236 studies were excluded from final review primarily due to lack of any identification of the allelochemicals present in the investigations or a quantifiable endpoint for germination/enzyme activity (e.g., ability of the extracted allelochemicals to induce the germination of seeds); however, many exclusions were also found to have examined the same data as another included report resulting in them being excluded from final analysis as well. Final analysis included a total of 116 studies, representing 3 inter-related theme clusters: chemical fingerprinting (n=34), germination

bioassays (n=48), and enzymatic/physiological mechanism studies (n=34).

3. Literature Review and Thematic Analysis

The development of this field is demonstrated in figure 2, which shows how many papers have been published on weed allelochemical fingerprinting and the effect these chemicals have on agriculture since 2019. There has been a large increase in the number of studies published during this time because of the ability to conduct untargeted metabolomics using GC-MS and LC-MS in plant science laboratories for the first time.

The three identified thematic areas from the published studies are shown in figure 4 and can be described as (i) chemical fingerprinting of weed allelochemicals (ii) effects of weeds on the germination stage (iii) effects of oxidative stress on enzyme activity). Each of these themes will be discussed in detail below.

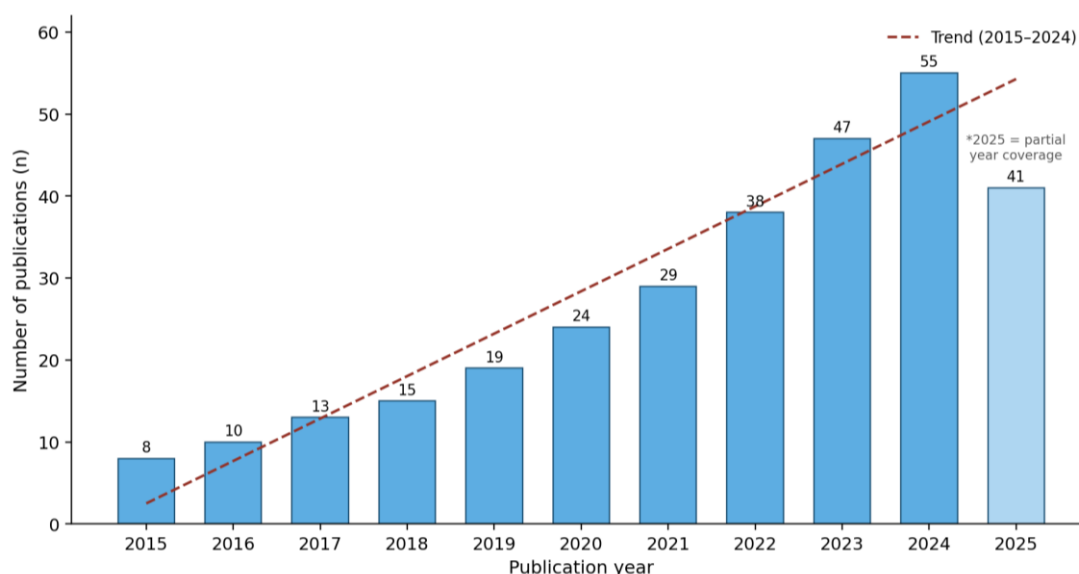


Fig 1: Publication trend on allelochemical fingerprinting of invasive weeds and crop physiological interference (2015–2025).

3.1. Theme 1: Analytical Fingerprinting of Weed Allelochemicals

Instead of identifying all components of plant extracts, metabolomic fingerprinting can identify the "chemical signature" of the plant extract but cannot provide a full profile of all metabolites in the plant. The primary method for metabolomic fingerprinting is gas chromatography-mass spectrometry (GC-MS) which is capable of detecting and reproducing the chemical signatures and is compatible with derivation processes that make it possible to separate and detect phenolic compounds that occur in both polar and non-polar states. There are many examples of using metabolomic fingerprinting for invasive plants, such as identify 22 phenolic compounds in leaf extracts of *Miscanthus sacchariflorus* (orientin, luteolin, chlorogenic acid, protocatechuic acid, p-coumaric acid, ferulic acid) using LC-MS/MS; and using stable isotope labeling of methyl palmitate and methyl linolenate to trace the relative translocation and allelopathic potentiality of these ester fatty acids across the plant (roots, stems, and leaves) using ^{13}C labelled tracers.

Profiling invasive cocklebur *Xanthium strumarium* by GC-MS showed changes in the amount of certain types of volatile organic compounds based on pathogen and herbivore pressure. This indicates that the chemical signatures of plants (i.e., their allelochemicals) can vary due to factors such as

biotic (living) and abiotic (non-living) interactions, and therefore, may not accurately represent how much of those compounds were released into field soil after they have been transformed (i.e., through microbial activity), leached (i.e., with water), or vaporized (i.e., escaped into the atmosphere) during those same periods. Caution should be taken when comparing chemical signatures from undamaged plants grown in greenhouses (i.e., glasshouse significant) to chemical signatures present in field soils as most allelochemicals are not static in plant tissues and have probably undergone significant alterations subsequent to release into the environment. Vegetable crop allelochemical fingerprints may become easier to obtain with as improvements in analytical methods yield better resolutions of allelochemical fingerprint data. Currently, weed science laboratories (in contrast to laboratories involved with other types of studies such as pharmacognosy or food science) use less accurate analytical methods to obtain weed allelochemical fingerprints. Advancements in analytical technology such as GC-TOF-MS [19] and GC-MS/MS [20], as well as improvements made by utilizing a combination of GC-MS and UPLC-QTOF-MS to generate an overall vegetable crop chemical profile [22] will likely improve the quality and quantity of plant allelochemical fingerprint data collected from the same plant samples.

While phenolic compounds (ferulic, p-coumaric, gallic, vanillic, caffeic and chlorogenic acids), sesquiterpene lactones (parthenin in *Parthenium hysterophorus*), lipid benzoquinones (sorgoleone from *Sorghum halepense*) and fatty acid esters have emerged as the compromise moieties of bioactivity^[4, 10, 11] across the literature to date, the majority of fingerprinting studies have only identified the individual targeting compound(s) that was detected not paired to an individual corresponding bioassay with the plant material and extraction batch that was used when establishing that particular compound(s) as being responsible for an observed biological response(s) as opposed to synergizing with other still unidentified bioactive moieties.

3.2. Theme 2: Interference with Crop Seed Germination

3.2.1. Species-specific germination bioassays

Out of 48 studies published about allelopathic influences on crops, the majority (n=48) used aqueous or organic extracts from invasive plants collected from either the leaves, roots, or stems (whole plant) of the invading weeds. Therefore, the number of studies that investigated plants and crops growing together has been substantially limited; for example, species of invasive plants, such as *Parthenium hysterophorus*, have been investigated more than any other species. For instance, in the wheat (*Triticum aestivum*), chickpea (*Cicer arietinum*), and mustard (*Brassica campestris*) plant studies by Hassan *et al.*^[10], increasing concentrations of leaf extract progressively reduced the rates of germination/shoot length/dry matter accumulation in those plants with the increase of concentration of the extract. The same pattern of concentration and response was also seen within maize plants' use of stem and leaf extracts at a rate of 15 g/L reduced the rate of germination to as low as 43.8% from 98.8% at the control (no extract), while the root elongation was suppressed much more effectively than the shoot elongation^[13], which shows that the root meristems were particularly sensitive to the allelochemicals present. The development of the current knowledge basis regarding the inhibition of crops by *Parthenium* occurs based on the underlying research completed by Kanchan and Jayachandra, who established that allelochemicals were released via root exudate^[11] and from leaching of the aerial vegetative components of the plant^[12], indicating that allelochemicals are delivered via multiple mechanisms, at multiple times, rather than through a single release process, to nearby crop seeds.

Mikania micrantha leaf aqueous extract inhibited seed germination more than it inhibited seedling growth from 26 native and naturalised species tested in South China, with non-legume species being more affected than legume species and native species being more affected than naturalised species^[5]. Therefore, legume seed coats or other biochemical mechanisms may allow legumes to have some degree of allelopathic tolerance to *Mikania micrantha* extracts. Similar results were found for the two invasive Lamiaceae (*Hyptis suaveolens* and *Leucas cephalotes*), which were both shown to affect aromatic rice with *H. suaveolens* having a greater effect than *L. cephalotes* on both germination and initial seedling growth^[8].

3.2.2. Root-parasitic and specialised germination interactions

A unique set of research provides functional insight into the allelochemicals produced by the invasive plant genus *Conyza* and their effects on parasitic broomrape weed (*Orobanche* and *Phelipanche* spp.) germination. Metabolite analysis of *Conyza*

bonariensis revealed two semi-structured stimulator compounds — hispidulin and methyl 4-hydroxybenzoate — with a shared ability to stimulate germination based on plant host species, as well as two lactone compounds, 4Z-lachnophyllum Lactone and 4Z,8Z-matricaria lactone, which inhibited size and growth of parasitic plant structures in radicle development regardless of the host species.

It is thus emphasised that the combined stimulatory or inhibitory properties of a donor plant can provide a clearer conceptual framework regarding the relative variability associated with allelochemical mixtures' phytotoxic influences than isolated compounds' phytotoxic influences.

3.3. Theme 3: Enzymatic and Oxidative-Stress Mechanisms

3.3.1. Hydrolytic enzyme suppression during imbibition

Enzymatic mobilisation of stored seed reserves allows germination to begin; the two main enzymes responsible are α -amylase which mediates starch hydrolysis and protease which helps break down protein. These breakdown products, soluble sugars, and amino acids, provide nutrients to the embryo as it germinates. Phenolic allelochemicals such as ferulic, p-coumaric, and other hydroxycinnamic acids commonly found in weed fingerprints have been shown to inhibit the activity of α -amylase in treated seed and reduce both the amount of available energy and carbon needed for embryonic development, which therefore lowers the percent of germination and extends the time required for radicle to emerge, even if seed has experienced no visible impairment due to imbibition.

3.3.2. Antioxidant enzyme perturbation and oxidative stress

There is also a second line of evidence that provides support for ROS accumulation and antioxidant enzyme disruption as a primary mechanism of allelochemical phytotoxicity. For example, during normal germination, superoxide dismutase (SOD) converts superoxide radicals to hydrogen peroxide. Then catalase (CAT) and peroxidase (POD) assist with the subsequent detoxification of this hydrogen peroxide^[23, 24]. When weed-derived allelochemicals are present, they disrupt this coordinated defence mechanism and characterized by a decrease in plant height, dry weight and chlorophyll content, a concurrent increase in H₂O₂ levels and significantly elevated levels of SOD, CAT, and POD activity were observed in *Trifolium repens* exposed to *Solidago altissima* root extract, with both the observed increases in enzyme activities, and the increases in H₂O₂ levels occurring in a dose dependent manner. Therefore the increases in enzyme activities are a compensatory response under the sub-lethal allelochemical stress from the weed. In another example, *Miscanthus sacchariflorus* phenolic-rich extracts produced altered rates of electrolyte leakage, photosynthetic pigment content and antioxidant enzymes in target weeds accompanied by suppressed germination and growth rates^[6], while *Parthenium* leaf-leachate growth stage — at the peak total phenolic and antioxidant activity in the donor tissue — produced the largest reductions in germination, shoot length, pod number and total biomass of cowpea (*Vigna unguiculata*), among all growth stages tested^[10]. Both of the previous studies demonstrated that exposure to allelochemicals results in comparable alterations in the activity of antioxidant enzymes in *Phalaris* weeds and *Eucalyptus*, highlighting the fact that the alteration of antioxidant enzyme activities is a common characteristic of allelopathy in different donor and target species. However, the

nature of the changes in antioxidant enzyme activity is not consistent among experiments. Some studies describe a compensatory increase in the activity of these enzymes at moderate concentrations of allelochemicals, while others relate the loss of antioxidant capacity and failure of the antioxidant defence system to the phytotoxic effects of high concentrations of allelochemicals as determined by the accumulation of malondialdehyde and the extent of electrolyte leakage. This apparent contradiction may best be explained by the expectation that there is a biphasic dose and duration response; therefore, antioxidant enzymes may be upregulated in response to allelochemical exposure at moderate concentrations but may be overwhelmed by the effects of higher or prolonged exposure to the allelochemicals. This pattern of response has been documented in many studies outside of allelopathy but has not been explicitly examined within one study of allelopathy that uses a broad range of concentrations and multiple sampling time points.

3.3.3. Molecular target sites: the sorgoleone paradigm

Sorgoleone is the most extensively understood chemical involved in allelopathy. It is a lipid benzoquinone that is produced in the roots of johnson grass and cultivated species of sorghum. Sorgoleone works by disrupting photosynthetic electron transfer at photosystem II. It does this by displacing the plastoquinone QB from the D1 protein. This mechanism of action is similar to that of the herbicide DCMU (where it acts as a competitive inhibitor). Sorgoleone also disrupts the electron transportation chain located in the mitochondria. Sorgoleone effectively inhibits the electron transport chain at

the cytochrome b-c1 complex and does so in both state 3 and state 4 respiration in isolated mitochondria at low concentrations (micromolar range).

The initial view of sorgoleone's mechanism of action as acting on both photosynthesis and respiration has been contested due to new evidence, which shows that older, intact plants are less responsive to root-applied sorgoleone than predicted from chloroplast bioassay. Consequently, new or alternative hypotheses regarding the mechanism of action of sorgoleone have been suggested. Such hypotheses postulate that sorgoleone primarily inhibits root plasma membrane H^+ -ATPase activity; thus, water uptake in plants is inhibited by sorgoleone^[29, 27].

A recent integrated synthesis has now reconciled these positions and proposed that sorgoleone is a true multitarget phytotoxin, acting as such by concurrently disrupting mitochondrial respiration; PSII electron transfer; carotenoid biosynthesis; and root H^+ -ATPase activity depending on tissue type (exposure), plant development (stage of maturity), and concentration^[30, 31].

The emerging details of these actions were refined progressively during about three decades, demonstrating the strengths and limitations of the field. There has been a very limited amount of mechanistic resolution to this detail for just a few well-investigated allelochemicals; the vast majority of the currently-existing compounds catalogued by fingerprinting studies (Section 3.1) have never been investigated to the same level of target-identification resolution.

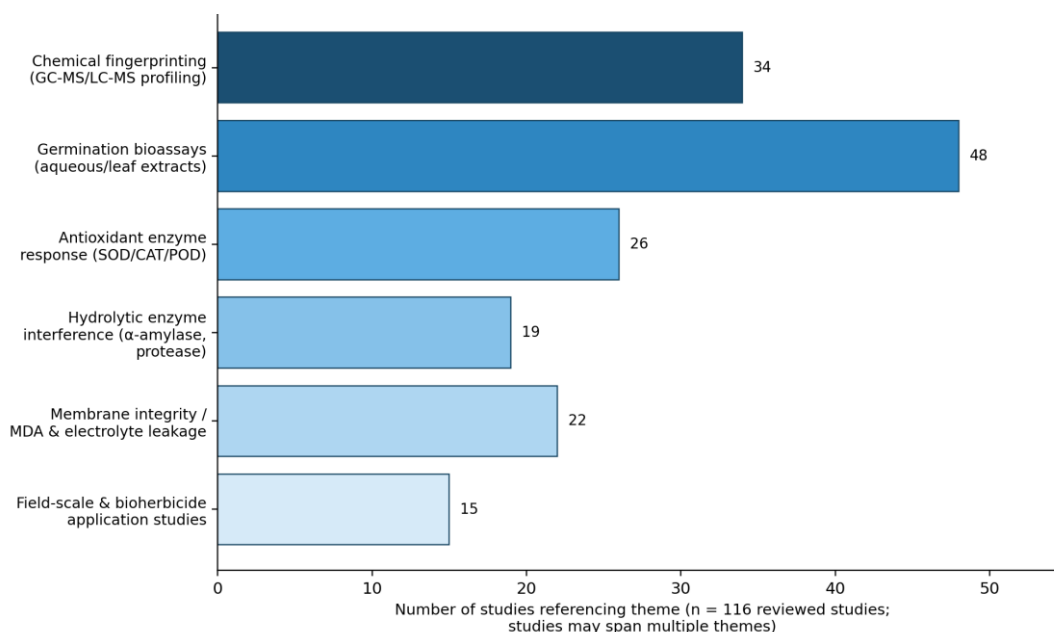


Fig 2: Thematic classification of reviewed studies (n = 116; studies may contribute to more than one theme).

3.4. Cross-Cutting Observations: Agreements, Contradictions and Practical Implications

The literature reviewed across the three topics also states, (i) the inhibitory effect of phenolic acids and terpenoid/quinone allelochemicals on hydrolytic and photosynthetic/respiratory enzyme activities is concentration dependent in a broad sense, (ii) germinating crop tissue is much more sensitive to these inhibitory effects than later developmental stages. Disagreement exists regarding the direction and magnitude of antioxidant enzyme responses to

sub-lethal exposures, the relative importance of photosynthetic vs. membrane-transport processes affected by quinone-type allelochemicals in planta, and the extent to which laboratory extract concentrations are similar to field-exposure scenarios. Practically, the aggregation of data supporting the reproducibility of the measurement of α-amylase and antioxidant enzyme inhibition serves as a viable basis for rapid allelopathic-risk screening of newly naturalised weed species, while the sorgoleone case study illustrates the feasibility of developing specific target molecules for the development of bioherbicides.

4. Comparative Analysis of Previous Studies

Table 3 summarises representative studies selected to span the major invasive weed genera and methodological approaches identified in the thematic synthesis, while Table 4 draws out cross-study methodological comparisons and limitations relevant to evidence quality.

5. Research Gaps and Emerging Trends

5.1. Unresolved Issues and Knowledge Gaps

Many unresolved problems exist within the reviewed literature. First, the causal relationship between the determined allelochemical fingerprint and the specific enzymatic disruptions that occur as a direct result of the allelochemical has rarely been demonstrated in a single study. Oftentimes, the fingerprinting and bioassay work are done on separate batches of samples (or separate studies altogether) and therefore undermine the ability to make causal attribution. Secondly, the biphasic (i.e., stimulation followed by inhibition) enzyme response that was inferred from the literature reviewed (in Section 3.3.2) has rarely been explicitly tested with enough of a concentration gradient and over multiple sampling times within the same experiment. Thirdly, nearly all of the bioassay work has been conducted using extracts from Petri dishes or pots in a laboratory environment, rather than calibrating the concentrations of the extracts against those found in the soil and rhizosphere of the field; thus, there are many valid reasons to question how applicable the reported effect sizes are in a field situation. Fourthly, the contribution of soil microbiomes to the transformation, degradation or potentiation of allelochemicals derived from weeds before they reach crop seeds has not been adequately examined in the literature pertaining to germination/enzymatic interference, even though other studies have acknowledged that allelochemicals can alter soil pH, the composition of the microbial community and organic matter dynamics.

5.2. Methodological Limitations

The reviewed studies show the presence of methodological limitations (see Table 4), such as: small replicate numbers; inconsistent reporting of concentration (mg mL⁻¹, molar concentration or w/v percentage); short period of observation; and a lack of multi-omic integration (e.g., metabolomic analysis combined with transcriptomic or proteomic analysis of exposed crop tissue). These limitations prevent understanding if the change in the enzymatic activity is a result of changing gene expression, through some form of post-translational modification or by way of direct chemical inhibition of the catalytic site on the enzyme.

5.3. Emerging Technologies and Future Developments

In future research, we expect to see a strong emphasis on combining untargeted metabolomics with transcriptomic and proteomic datasets to develop multi-omics models that illustrate the mechanism by which allelochemicals work; machine-learning models will be used to identify allelochemical–enzyme interactions and help select which allelochemical chemical to isolate and test for biological activity; advances in nanotechnology research will lead to the development of encapsulated and controlled-release formulations using allelochemicals; and there will be an increase in collaboration with researchers studying the rhizosphere microbiome to provide a better understanding of how allelochemicals break down in the soil environment. Emerging trends related to these areas are shown in Table 6,

which also lists the expected value of these trends as future research directions. Figure 6 shows the expected directions of future research.

6. Discussion

Combining what we can learn from all the available literature on how weeds chemically inhibit crop and other plants from germinating gives us a general but still a partial understanding of how certain 'weeds' can affect crop germination through their release of allelochemicals via root exudation, leaf leachates, volatilisation, and decomposed residues. These allelochemicals all converge on two major physiological processes during seed germination: reservoir mobilisation (by inhibiting α -amylase and protease activity) and controlling redox status (by producing species reactive oxygen and inhibiting antioxidant enzymes). The fact that phenolic acids, sesquiterpene lactones, and quinone-type allelochemicals have consistently been implicated in the subtle and distinct differences between taxonomically unrelated invasive weed species (e.g., from *Parthenium* spp of South Asia through to *Sorghum halepense* of temperate cropping to *Mikania* of subtropical China) indicates that these invasive plants are likely using similar biochemical strategies rather than having developed their idiosyncratic pathways. Based on this evidence, allelopathy appears to represent a form of adaptation that has been widely selected for and conserved as part of the competitive success of invasive plants.

Simultaneously, the field also shows a quality gradient of evidence, which parallels the evolution of the field. For the relatively few compounds that have been extensively studied, such as sorgoleone, there has been three decades of incremental research leading to the development of a detailed multi-target mode of action. On the contrary, the majority of recently fingerprinted compounds, from the less-studied invasive genera of plants, are still reported as only having extract based bioassay identification, and no confirmed single compound activity or specific molecular targets. This creates a translational bottleneck; the rate of advancement in technology for chemical fingerprinting has exceeded the rate of advancement in mechanistic bioassay research needed to convert fingerprint data into usable biomarkers or bioherbicide leads.

The apparent contradiction about the directionality of antioxidant enzyme activity (up-regulated versus collapsed) is best interpreted as not being contradictory per se, but rather indicating that they are both manifestations of dose and time dependent biphasic toxicology that has not been systematically mapped across numerous independent and well-controlled experiment systems, covering a wide concentration range of the active toxicant and multiple sampling points. Addressing this would likely significantly improve the database of evidence and provide clarity as to whether induction of antioxidant enzymes is an exploitable early warning biomarker of sub-lethal allelochemical stress in field-grown crops and thus is practically useful for the precision monitoring of weed risk.

In theory, they support the hypothesis that allelopathy is an integral part of invasive weed competitive strategies instead of an unintentional, non-essential result of secondary metabolism which has important implications for invasion biology, integrated weed management and policies regarding sustainable agriculture. From a practical standpoint, they provide a reproducible basis to produce rapid diagnostic bioassays to identify new naturalized weed species that pose a high-risk of allelopathy, as well as help isolate candidate

natural-product bioherbicides that have specific modes of action. This would also be increasingly important, given that regulations and consumer demand are calling for a decrease in dependence upon synthetic herbicides.

7. Conclusion

The present review presents a synthesis of evidence from 116 peer-reviewed studies regarding the chemical fingerprinting of allelochemicals from invasive weed species and biochemical interference with crop seed germination and enzymatic metabolism. This body of work reveals that phenolic acids, sesquiterpene lactones, quinones and fatty-acid esters are predominant bioactive chemical subclasses across invasive weed species that have global significance. Allelochemicals belonging to these classes consistently correspond with inhibited hydrolytic enzyme activity, increased reactive oxygen species (ROS), and altered oxidant systems (antioxidant enzyme systems) in exposed crop seeds and seedlings, ultimately resulting in a reduced germination percentage, reduced seedling vigour and decreased establishment of seedlings in crop production systems.

The significance of this collective body of research is that it provides evidence that allelopathy is not merely an ecological descriptor but rather a tractable chemical phenomenon that can be mechanistically analysed. This development has opened the door to improved risk assessment processes concerning newly emerging invasive species as well as opportunities for the rational design of selective, plant-derived bioherbicides. However, methodological variation and a predominance of laboratory-based (bioassay literature) methods that do not reflect field conditions, have hampered progress toward these ends. Additionally, the current body of work has limited integration of multi-omics and/or microbiome approaches into existing research frameworks.

Both researchers and practitioners / policymakers engaged in invasive species management and sustainable crop protection should give priority to the following: coupling chemical fingerprinting with confirmatory single compound bioassays; reporting concentrations based on standardised metrics; and using experiments that can differentiate between biphasic dose–time enzyme responses. The body of evidence reviewed suggests that allelochemical risk screening can be incorporated into the early warning framework for newly naturalised weeds and provides reason to continue investing in field validated bioherbicides that are based on mechanisms established by current findings. Future research should prioritise: field scale validation; the integration of mechanistic data through the use of multi-omics approaches; the investigation of rhizosphere microbiome dynamics; and studies on interaction effects due to climate change (see Section 5.3). This will aid in converting major findings related to chemistry and physiology into tangible tools for sustainable management of weeds.

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