

MICROBIAL ALLELOPATHY: A REVIEW ON ECOFRIENDLY AND SUSTAINABLE WEED MANAGEMENT STRATEGY

RAJAKUMAR, D.¹ – GOMATHY, M.^{2*} – SABARINATHAN, K. G.^{3*}

¹*Department of Agronomy, VOC Agricultural College and Research Institute, Killikulam
628252, Thoothukudi, Tamil Nadu, India
(e-mail: ds.rajakumar@gmail.com)*

²*Department of Soil Science and Agricultural Chemistry, VOC Agricultural College and
Research Institute, Killikulam 628252, Thoothukudi, Tamil Nadu, India*

³*Department of Agricultural Microbiology, Agricultural College and Research Institute,
Madurai 625104, Tamil Nadu, India
(e-mail: sabarimicro@hotmail.com)*

**Corresponding authors
e-mail: gomathymicro@gmail.com; sabarimicro@hotmail.com*

(Received 29th Jun 2024; accepted 18th Oct 2024)

Abstract. The role of microbes and their allelochemicals as microbial herbicides in weed management has been explored in this article, highlighting their types, benefits and associated challenges. It emphasizes the importance of alternative biological weed control methods amidst increasing herbicide resistance and outlines the potential of microbial/PGPR allelochemicals as bioherbicides. The significance of microbial allelochemicals in promoting sustainable agriculture cannot be overstated. As the exploration of these natural compounds progresses, it is imperative to focus on their integration into current agricultural practices. Through detailed analysis and comparative evaluation, the article provides a roadmap for future research and development in this field. This approach not only aligns with the global efforts towards environmental sustainability and reduced chemical dependency but also opens avenues for innovative weed management strategies that are both effective and ecologically responsible. This overview aims to lay the groundwork for further exploration and adoption of microbial allelochemicals in effective weed management strategies globally.

Keywords: *microbial allelochemicals, bioherbicides, biological weed management, mycoherbicides, ecofriendly weed management*

Introduction

Weeds pose significant challenges in agriculture by competing with crops for essential resources such as water, air, nutrients, space and light. Additionally, weeds can serve as alternate hosts for pests and diseases (Swanton et al., 2015; Ramesh et al., 2017). The extent of crop yield losses due to weeds varies considerably, depending on factors such as the type of crop, methods of weed control, weed species, fertilizer management practices and abiotic factors like climate and soil conditions (Kaur et al., 2018). Due to labor shortages in agriculture, the global use of herbicides for weed control has increased. Manual weeding remains effective for small-scale farms due to the limited area and the feasibility of managing weeds manually with fewer labor resources. However, for large-scale farming, manual weeding becomes economically and operationally impractical. The labor requirement for manual weeding varies significantly depending on the crop, soil conditions and the time of weeding. For staple crops like rice or maize, manual weeding can demand up to 150-300 h per hectare, depending on weed density (Singh et al., 2017). Similarly, manual weeding can require

as much as 250-780 h per hectare, as observed for upland rice (Bergman Lodin et al., 2012; Ogwuiké et al., 2014). In maize, using pre-emergence herbicides followed by hand-weeding reduced labor to 18 man-days per hectare, compared to 45 man-days for exclusive hand-weeding (Mynavathi et al., 2015). While herbicides reduce labor inputs, they are not yet widely adopted in subsistence farming systems in Africa (Gianessi, 2013) and organic farmers realized costs of up to 2500 USD per hectare for weed control (Gianessi and Reigner, 2007). Properties of weeds like deep root system, resistance to biotic and abiotic stress tolerance and high resource use efficiency make them competitive resulting not only in yield reduction but also increase in cultivation cost (Trognitz et al., 2016). To mitigate crop yield losses due to weeds, which pose a significant challenge to agricultural productivity worldwide, the exploration of effective weed management strategies is imperative.

Aside from fungicides, herbicides are the primary chemical compounds utilized in agriculture, accounting for over 40% of the pesticides used globally (Heap, 2015). Indiscriminate use of synthetic herbicides, acquiring of herbicide resistance by weeds and environmental concerns related to some herbicide usage necessitates the development of ecofriendly efficient herbicides with relatively low residual toxicity and decreased toxicity to non-target organisms with novel molecules and sites (Reddy and Nandula, 2012; Heap, 2015; Sondhia and Singh, 2018). Undeniable concern on environment paved way for research studies on bioherbicides and their phytotoxic metabolites have been on the go in many countries. Weed-suppressive and allelopathic compounds can be extracted as natural products from microorganisms and plants. For decades, research has concentrated on utilizing bacteria and fungi to control unwanted plants. These microbial agents or their compounds, known as “bioherbicides,” suppress weeds through plant-pathogen interactions or allelopathy. Microbial bioherbicides are naturally derived compounds from microorganisms such as fungi, bacteria, viruses and also plant species including weeds. Biological control of weeds include inoculative (classical) and inundative (augmentative) methods. In the classical approach, virulent host specific pathogens feed on weeds, reproduce and suppress them. The biocontrol organism builds up a population to an extent that could manage the weed. The introduced population is maintained over very long periods of time (Bale et al., 2008). Contrary to inoculative method, in augmentative approach, isolated pathogens from weeds after inoculation damage the weeds in a phased manner (Ani et al., 2018; Kumar and Agarwal, 2024). Though mode of application of weed pathogens is similar to herbicide applications, viability needs to be maintained to achieve satisfactory results.

The diversity of allelopathic chemicals makes them promising tools for targeting specific sites in plants, offering a way to eliminate weeds resistant to current herbicides. Unlike synthetic herbicides, allelochemicals act on multiple sites in plants and lack high specificity. However, their effects are highly dose-dependent, which opens the possibility of finding compounds that can be selective. Typically, monocotyledonous plants are more resistant to allelochemicals than dicotyledonous ones (Soltys et al., 2013). Therefore, using allelopathic compounds as herbicides is feasible but limited to specific crops and weed compositions. Among these strategies, the use of allelochemicals, particularly those derived from microbial sources or Plant Growth-Promoting Rhizobacteria (PGPR) as bioherbicides, has emerged as a promising alternative to conventional herbicides (Sindhu et al., 2018; Adetunji et al., 2019). This shift is driven by the increasing concern over herbicide resistance and the environmental impact of synthetic chemicals used in agriculture. Microbial allelochemicals offer an

eco-friendly and sustainable solution for controlling weeds, heralding a new era in the pursuit of not only enhancing crop yield but also ensuring environmental health.

Microbial allelochemicals

Microorganisms that are present naturally in the rhizosphere possess the ability to suppress weed growth by infecting the root surfaces of weed seedlings and inhibiting their growth and reproduction thereby reducing weed density and biomass (Adetunji et al., 2019). Research shows that these microorganisms can effectively change the competitive dynamics within the rhizosphere, significantly decreasing weed proliferation and offering a sustainable approach to weed management (Dahiya et al., 2019).

Plant roots exude different organic nutrients and signals unique to the microbial populations to attract and utilize those plant exudates for their metabolic processes. Rhizosphere is the hub of microbial diversity and the composition of root exudates determine the microbial community often referred as rhizo-microbiome. The rhizosphere microbiome is heterogenous in time and space as the root exudate composition varies according to plant species, varieties and even growth stages (Mohanram and Kumar, 2019; Sindhu et al., 2017; Lareen et al., 2016).

Similarly, PGPR produce wide range of secondary metabolites called allelochemicals like siderophores, antibiotics, biocidal volatiles, lytic and detoxification enzymes that indirectly enhance plant growth through suppression of phytopathogens. The direct and indirect role of PGPR as contributors for plant nutrition through biological nitrogen fixation, phosphate solubilization and phytosiderophore production has been recognized globally. PGPR if available in adequate population provide an absolute rhizosphere for plant growth and converting nutritionally important elements available for plants. Consonant to the above, exploring the possibilities of utilizing rhizosphere microbes as bioherbicides has been the priority nowadays.

Microbial allelochemicals encompass a diverse range of chemical compounds produced by microorganisms that influence plant growth and development. These natural constituents play crucial roles in plant interactions, serving both defensive and communicative functions in ecosystems. Allelochemicals, including phenolic compounds, terpenoids, alkaloids and various nitrogen-containing chemicals, are primarily involved in the defense against microbial attacks, herbivore predation and competition with other plants (Kong et al., 2019). Their actions are evident in phenomena such as allelopathy, where they inhibit the establishment of competing plants through chemical interference.

The concept of allelopathy and the role of allelochemicals in plant interactions have been recognized for centuries. Over the years, the identification and understanding of these compounds have evolved significantly. Recent research has expanded the knowledge on roles of allelochemicals, where they offer potential for natural weed management and pest control (Hoagland, 2001). Advances in technology have enabled the detailed study of these compounds, including their molecular mechanisms and ecological impacts. The study of allelopathic phenomena involves understanding the biochemical interactions among various plants, including vascular plants and microorganisms. These interactions can have either positive or negative effects on plant growth and development. Allelopathy covers all such interactions that influence plants. Biological control refers to using living organisms to reduce or suppress pest populations.

An alternative to utilizing living microorganisms for weed control is the application of compounds they produce, known as “natural products.” These compounds, which possess weed-suppressive and allelopathic properties, can be isolated from microorganisms. Such natural products offer a sustainable and potentially less disruptive option for weed management compared to traditional chemical herbicides (Katz and Baltz, 2016). In this context, bioherbicides are biological agents, such as fungi, bacteria and algae, applied to plants to control weeds. They can also include secondary allelochemical products produced by microbes or plants, utilized for weed management (Hoagland, 2001). The exploration of microbial allelochemicals is part of a broader effort to harness nature’s own strategies for managing agricultural challenges. By understanding and applying the principles of allelopathy and microbial interactions, researchers aim to develop sustainable practices that reduce reliance on synthetic chemicals and enhance crop productivity.

Types of microbial allelochemicals

Bioherbicides are natural herbicides derived from plant extracts as well as living organisms such as bacteria, fungi, and viruses, or from the secondary metabolites these organisms produce during their growth and development. At present, the use of viruses as bioherbicides is limited because of their high genetic variability and unstable host specificity, making them less reliable for consistent weed control (Cordeau et al., 2016). Microbes use enzymes, peptides and secondary metabolites with phytotoxic properties to overcome the resistance barriers of weed plants and fully infect the weeds making them potent bioherbicides. These virulence factors include enzymes such as amylases, pectinases, cellulases, lignin-modifying enzymes, proteases, peptidases and phospholipases, which break down the cell walls, lipid membranes, and proteins of weed plants. The peptides and secondary metabolites consists of hydrogen cyanide (HCN), ethylene, ammonia, dimethyl disulfide, indole-3-acetic acid, hydrocinnamic acid and aminolevulinic acid (Radhakrishnan et al., 2018).

Root-colonizing bacteria can be categorized into plant growth-promoting rhizobacteria (PGPR) or deleterious rhizobacteria (DRB). These include allelopathic or nonpathogenic or phytotoxic bacteria which are selective and specific to individual root zone of plants and can affect plant growth positively or negatively and directly or indirectly (Kremer and Kennedy, 1996; Kennedy and Stubbs, 2007). Allelopathic non-pathogenic bacteria secrete a diverse allelochemicals and some species produce multiple allelochemicals. Though production of rhizobial exudates and related microbiome is plant driven, the influence of allelopathic bacteria on higher plants differ in their target specificity. Still, many of the *Pseudomonas* sp. are found capable of producing antibiotics that are selectively detrimental to weeds. This has provided a new insight into the invention of many bioherbicide molecules and newer site of actions. Similarly, DRB can colonize weed root surfaces and effectively suppress weed growth thus giving crops an edge over the weeds (Kremer and Kennedy, 1996).

Bacterial allelochemicals are produced by various bacterial species, particularly those associated with plant roots, known as rhizospheric bacteria. These compounds include various antibiotics, siderophores and volatile organic compounds that inhibit the growth of competing plant species. *Pseudomonas* can produce significant amounts of HCN and limits weed growth. For instance, certain strains of *Pseudomonas* and *Bacillus*

produce substances that suppress pathogenic fungi and inhibit weed seed germination, thus aiding in crop protection and yield enhancement (Phukan et al., 2021).

Streptomyces spp. are a major group of inhabitant bacteria often proved to have compounds with herbicidal activity. Monensin, a carboxylic polyether isolated from *Streptomyces cinnamonensis* inhibit the protein secretion through its effect on golgi apparatus (Hoagland, 2001). Nigericin, isolated from *Streptomyces hygroscopicus* was found to be phytotoxic to several weeds as pre and post emergence application. Researches leading to the development of bacterial herbicides were initiated since 1990s and lack of strong and precise herbicidal activity was a major impediment to large scale adoption. The discovery of phytotoxic allelochemicals bialaphos and phosphinothricin isolated from *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus* cultures sustained the scope of developing PGPR based bioherbicides. It is marketed under a number of trade names including Glufosinate, Basta, Challenge, Finale and Radicale which are non-selective herbicides. Non-selective microbial commercial herbicides target a broad spectrum of weeds, offering an alternative to chemical herbicides, yet they face critical limitations. Products from *Phoma macrostoma* (BioMal) show promise in controlling various weed species, but their efficacy can be inconsistent under field conditions due to environmental factors such as temperature, humidity and soil composition (Boyetchko et al., 2002). Additionally, these herbicides often lack the precision of selective synthetic herbicides, which limits their use in crops. Moreover, non-selective microbial herbicides may affect both target and non-target plant species, potentially reducing crop safety (Bailey et al., 2011). Thus, while offering ecological benefits, they require improvements in formulation and application techniques to enhance reliability and effectiveness.

Fungi inhibit the growth and development of weeds by producing secondary metabolites called “mycotoxins” belonging to amino acids, coumarins, isocoumarins, terpenes, phenols, steroids, xanthenes, quinones, terpenoids, alkaloids, polyketones, flavonoids and benzopyranones which may be host specific (selective) and non-specific (non-selective) toxins (Nisa et al., 2015; Cimmino et al., 2015; Xu et al., 2021). Fungal pathogens used for weed control are commonly known as “mycoherbicides.” These mycoherbicides are a specific type of bioherbicide that employ fungi to target and suppress weed populations. When applied to weed plants, these fungal pathogens infect and often kill or significantly weaken the weeds, thereby reducing their growth and spread. This method leverages the natural antagonistic relationships between certain fungi and weed species, offering a biological alternative to chemical herbicides. Mycoherbicides are part of integrated weed management strategies aimed at achieving sustainable and environmentally friendly weed control.

Fungi are prolific producers of allelochemicals, with species like *Penicillium* and *Aspergillus* being notable for their ability to produce a wide array of mycotoxins and other bioactive compounds. These allelochemicals often possess strong inhibitory effects on plant pathogens and weeds. Fungal allelochemicals not only help in controlling agricultural pests but also play a crucial role in soil health by decomposing organic matter and recycling nutrients.

Each type of microbial allelochemical offers unique advantages and mechanisms for weed suppression, making them integral components of integrated weed management systems. Unlike synthetic herbicides, microbial allelochemicals reduce environmental pollution and minimize the risk of herbicide resistance, a growing issue with repeated chemical use. Weeds develop resistance through mechanisms such as target-site

resistance, where mutations reduce herbicide efficacy (Powles and Yu, 2010), and metabolic resistance, where weeds detoxify the herbicide or limit its uptake (Delye et al., 2013). In contrast, allelopathic compounds act through diverse biochemical pathways, making it harder for weeds to evolve resistance (Macias et al., 2007). With multiple modes of action, allelopathic substances exert less selective pressure on a single target, offering a sustainable alternative to chemical herbicides (Kostina-Bednarz et al., 2023).

Mechanism of action

The mechanism by which PGPR inhibit the growth of weeds and germination of weeds seeds varies with the production of plant growth promoting compounds, phytotoxins, antibiotics, IAA, ALA, HCN and volatile compounds (Mejri et al., 2010; Sindhu et al., 2018; Adetunji et al., 2019). Indole-3-acetic acid though beneficial at lower concentrations its effect is reversed with higher concentrations and thus lethal to plant growth and development. Being photodynamic, aminolevulinic acid serves as biodegradable herbicide (Phour and Sindhu, 2019). Rhizobacteria like *Pseudomonas* and *Bacillus* with their ability to produce HCN, inhibit root cell metabolism (Radhakrishnan et al., 2018).

Inhibitory effects on weeds

Microbial allelochemicals exhibit a range of inhibitory effects on weed growth and germination. An alternative bioherbicidal approach involves using phytotoxic allelochemicals produced by microorganisms, both pathogenic and nonpathogenic, to control weeds. Pathogens can harm plants by disrupting their metabolism through nutrient competition, enzyme production, and the release of phytotoxins. Many microorganisms produce multiple phytotoxins, which can collectively damage or kill plants. Despite their potential, the specific phytotoxins and their modes of action have not been fully explored.

Plants have their own defense mechanisms against pathogens, often producing allelopathic compounds to fend off attacks. In bioherbicide research, these natural phytotoxins are used directly or as models to develop synthetic analogs. Although many microbial phytotoxins have been identified and studied, their complex structures make them difficult to synthesize commercially. However, understanding their molecular action can help identify new herbicide targets. As a result of coevolution between the pathogen and its host plant, some phytotoxins are host-specific, affecting only the plant species to which the microorganism is pathogenic (Zeller et al., 2007; Kennedy et al., 2001).

Interaction with soil microbiome

The interaction between microbial allelochemicals and the soil microbiome plays a pivotal role in their mechanism of action. Allelochemicals released from root systems can significantly influence the microbial community in the soil, affecting soil properties and plant growth. For example, benzoxazinoids like DIMBOA (2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one) and DIBOA (2,4-dihydroxy-(2H)-1,4-benzoxazin-3(4H)-one) produced by cereals can modify the soil microbial community structure, particularly influencing fungi populations in cereal rhizospheres (Jilani et al.,

2008; Inderjit et al., 2005). Benzoxazinoids are synthesized in cereals and released into the surrounding soil solution through decomposition of plant tissues and residues, as well as root exudation from root hairs or secondary roots (Reiss et al., 2018). After their production and release, these compounds undergo physicochemical and microbiological changes, leading to alterations in their phytotoxicity mediated by microorganisms (Hussain et al., 2022). This interaction not only suppresses plant pathogens and herbivores but also enhances plant defense mechanisms. Moreover, soil microorganisms are crucial in the decomposition of allelochemicals, which can either enhance or reduce their allelopathic effects. Microbes can deactivate water-soluble phytotoxins or transform harmless compounds into phytotoxins, thus playing a dual role in moderating allelopathic activities (Xiao et al., 2020).

These interactions highlight the complex dynamics between microbial allelochemicals, the soil microbiome and plant health, underscoring the potential of microbial allelochemicals in sustainable agriculture and effective weed management strategies.

Benefits of microbial allelochemicals

Bioherbicides based on microorganisms are an alternative to chemical herbicides and these microbial allelochemicals present several benefits that are transforming agricultural practices towards more sustainable and environmentally friendly methods. These benefits are particularly evident in their role as eco-friendly alternatives and their contribution to sustainability in agriculture (Bordin et al., 2021; Pacanoski, 2015).

Microbial allelochemicals, often derived from plant growth-promoting microorganisms (PGPM), are pivotal in providing eco-friendly solutions to agricultural challenges. These bioinoculants, comprising living or dormant microbes, enhance plant growth and development while also offering a cost-effective and environmentally friendly approach to agriculture (Kong et al., 2019; Mushtaq et al., 2020; Perotti et al., 2020). The application of microbial allelochemicals supports sustainable agricultural practices by enhancing soil health and reducing the reliance on chemical herbicides. These natural compounds can boost seed germination, aid development and foster the growth of crop plants with minimal phytotoxic remains, facilitating the recycling process and wastewater treatment (Ain et al., 2023). Furthermore, microbial allelochemicals contribute to the development of resistance against various abiotic and biotic stresses in plants, promoting an economical and efficient method to improve crop productivity. The implementation of microbial bioherbicide technology within integrated weed management strategies in diversified cropping systems has the potential to significantly enhance soil fertility and productivity in degraded ecosystems due to abuse of synthetic chemicals. Since these natural phytotoxins have various modes of action, this approach can also prevent the development of herbicide-resistant and invasive weed species (Bordin et al., 2021; Hasan et al., 2021; Kremer, 2005). When microbial herbicides are effectively integrated into agricultural practices and environmental restoration efforts, they can play a pivotal role in reclaiming and restoring biodiversity to ecosystems that have been degraded by the persistent use of pesticides in crop cultivation (Sehrawat and Sindhu, 2019). By integrating these compounds into crop production, it is possible to achieve a reduction in the large-scale use of herbicides and to introduce organic production systems, thus meeting the increasing consumer demand for safe and superior agricultural products (*Fig. 1*).

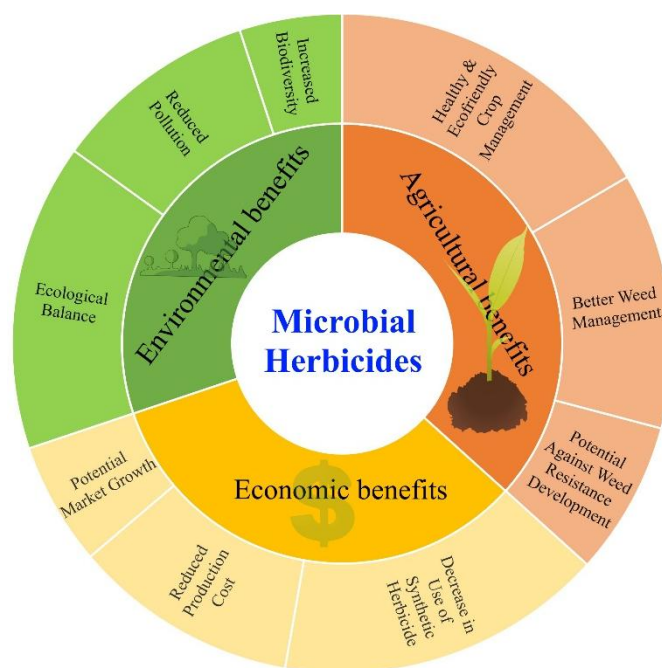


Figure 1. Potential agricultural, environmental and economic roles of microbial herbicides

The integration of microbial allelochemicals into modern agriculture not only enhances the ecological balance but also aligns with global efforts to ensure food security and environmental sustainability. Their role in modulating the soil microbiome and enhancing plant self-defense mechanisms underscores their potential as a cornerstone in the future of sustainable agriculture.

Challenges and limitations

Microbial herbicides, though promising as eco-friendly alternatives to chemical herbicides, face several challenges and bottlenecks that hinder their widespread adoption. These include the complexity of producing and scaling up large quantities of microbial agents, ensuring their survival in varying environmental conditions and the unpredictability of their behavior in the field. Factors like inconsistent efficacy due to diverse weed populations, dependency on specific weather conditions and difficulties in formulating stable products add further complications (*Table 1*). Addressing these challenges is essential for realizing the full potential of microbial herbicides in sustainable agriculture.

Allelopathic microbial herbicides are limited to specific crops and weeds due to their narrow-spectrum activity and sensitivity to environmental conditions. These herbicides rely on microorganisms that produce bioactive compounds, which typically target specific weed species while sparing others. The fungus, *Phoma macrostoma* controls broadleaf weeds like dandelion but is ineffective against grasses (Bailey et al., 2011). Similarly, *Myrothecium verrucaria* targets kudzu (*Pueraria montana* var. *lobata*) but has limited impact on other weeds (Boyette et al., 2002). Environmental factors such as soil type and moisture also influence their efficacy, restricting their broader application. Moreover, some microbial herbicides can harm non-target crops, further limiting their use. The bacterium, *Pseudomonas fluorescens* strain CL145A was effective against

Eurasian watermilfoil (*Myriophyllum spicatum*) but is not effective against other aquatic weeds (Jinnah et al., 2010). Thus, their selectivity and environmental dependence restrict their widespread use in agriculture.

Table 1. Challenges and limitations of mycoherbicide application in weed management

Challenges and limitations	References
Most research focuses primarily on a narrow group of microorganisms, especially fungi, while the vast majority of other microorganisms, such as bacteria and actinomycetes, remain largely unexploited	Cheng et al., 2022
Need for precise synchronization between the growth stages of the biocontrol microorganism and the susceptible period of the target weed. Achieving this synchronization requires a deep understanding of the life cycles of both the weed and the microorganism, along with extensive experimentation	Kennedy and Stubbs, 2007; Cliquet and Zeeshan, 2008
Weeds exhibit significant genetic variation, and a microbial strain that effectively controls one population may not perform as well against another	Ward et al., 2008
Before a biological herbicide can be commercialized, thorough host-range studies must be conducted to assess its impact on non-target organisms, ensuring minimal harmful effects. Failure to perform adequate host-range testing often results in the premature discontinuation of a promising biocontrol agent. This complexity makes the development of effective bioherbicides more difficult and time-consuming	Stubbs and Kennedy, 2012
Producing large quantities and ensuring the survival of microbial products: While bacterial herbicides offer advantages like simpler fermentation and easier mass production compared to mycoherbicides, maintaining viability and effectiveness during large-scale application remains a significant hurdle	Amsellem et al., 1999; Li et al., 2003
Optimizing technology and cultural practices: Factors like droplet size, spray direction and inoculum levels affect spray retention and efficacy, while wider row spacings often require repeated applications for effective weed suppression	Lawrie et al., 2002; Byer et al., 2006; Boyette et al., 2007
Lengthy and resource-intensive process of screening and testing isolates: Extensive additional testing and evaluation of host range, formulation, soil survival, production and application to satisfy both consumer demand and regulatory requirements	Cheng et al., 2022
The challenge with bioherbicide agents is their unpredictable behavior in soil due to interactions with varying environmental factors such as soil composition, moisture, temperature and competing microorganisms, which can impact their effectiveness	Lazarus et al., 2021

By integrating these natural compounds into crop production, it is possible to reduce the large-scale use of synthetic herbicides and support organic production systems, aligning with consumer demand for safer agricultural products. However, it is essential to recognize that natural compounds are not inherently risk-free and must be carefully assessed for safety and environmental impact before widespread use. These challenges underline the complexity of developing and implementing microbial herbicide-based weed management strategies. Further research and innovative approaches are required to overcome these limitations and fully harness the potential of microbes in sustainable weed management.

Future potential and research directions

Advances in genome sequencing allow scientists to identify new gene clusters responsible for producing important compounds. Studies have shown that microbial genomes contain many more of these gene clusters than previously thought, based on known compounds (Blin et al., 2021). To find useful natural products, deep learning methods are being developed to screen chemical libraries and better predict the functions of these compounds based on their structures. Because genomic datasets are usually large and complex, bioinformatic tools are used to sift through this data and find potentially valuable natural products (Skinnider et al., 2017; Stokes et al., 2020).

Furthermore, the development of targeted methods such as metagenomics and quantitative stable isotope probing is advocated to assess the impacts on microbiome functional responses and subsequent plant–microbial interactions. These innovative techniques hold promise for elucidating the intricate mechanisms through which microbial allelochemicals influence agricultural ecosystems.

The role of collaborative research in enhancing the understanding of microbial allelochemicals is undeniable. By combining efforts across disciplines, researchers can explore the differential responses of bacterial and fungal communities to allelopathy. This collaborative approach is essential for developing comprehensive models that predict the behavior of microbial interactions under various ecological stressors. Additionally, the integration of findings from different studies, such as the impact of microbial activity on the biodegradation of allelochemicals, provides a robust framework for future research. These collaborative efforts are crucial for advancing the application of microbial allelochemicals in sustainable agriculture and effective weed management.

Conclusion

In summary, while synthetic herbicides have contributed to increases in agricultural productivity, their long-term use raises concerns regarding environmental health and sustainability. Recent bioherbicides encompass a variety of microbial agents, including obligate fungal parasites, soil-borne fungal pathogens, non-phytopathogenic fungi and pathogenic and nonpathogenic bacteria. These organisms often have different cultural and application requirements compared to earlier mycoherbicides. This creates a unique challenge: although the number of potential bioherbicides and their target weeds in diverse habitats has increased over the past 25 years, the complexity of their production, formulation and application methods has also grown. Even then, Microbial allelochemicals, as part of an integrated weed management system, offer a promising alternative that supports ecological balance and reduces the chemical load in agricultural settings. The effectiveness of bioherbicides might be increased by combining them with adjuvants or formulations that can protect the survival and enhance the microbial herbicidal activity (Stubbs and Kennedy, 2012; Kubiak et al., 2022).

Another innovative approach that warrants research attention is the use of a mixture of pathogens and their allelochemicals to control weed growth, rather than relying on a single pathogen (Stubbs and Kennedy, 2012). This strategy, involves the combined application of multiple microbial agents, each with unique modes of action and target specificities (Caesar et al., 2010; Rayamajhi et al., 2010). By exploiting a diverse array of pathogens, the approach can enhance weed suppression efficacy, reduce the

likelihood of resistance development, and potentially offer broader spectrum control over various weed species. Additionally, the synergistic interactions among different pathogens can improve their overall performance and persistence in the field. This multifaceted method represents a promising direction for sustainable weed management, integrating biological diversity to achieve more effective and resilient control measures. The future of agricultural productivity hinges on our ability to embrace and enhance the use of microbial allelochemicals in a manner that benefits both the crops and the ecosystems they thrive in.

REFERENCES

- [1] Adetunji, C. O., Oloke, S. A. S., Bello, O. M., Pradeep, M., Jolly, R. S. (2019): Isolation, structural elucidation, and bioherbicidal activity of an eco-friendly bioactive 2-(Hydroxymethyl) phenol from *Pseudomonas aeruginosa* (C1501) and its ecotoxicological evaluation on soil. – *Environmental Technology & Innovation* 13: 304-317.
- [2] Ain, Q., Mushtaq, W., Shadab, M., Siddiqui, M. B. (2023): Allelopathy: an alternative tool for sustainable agriculture. – *Physiology and Molecular Biology of Plants* 29(4): 495-511.
- [3] Amsellem, Z., Zidack, N. K., Quimby Jr. P. C., Gressel, J. (1999): Long-term dry preservation of viable mycelia of two mycoherbicidal organisms. – *Crop Protection* 18(10): 643-649.
- [4] Ani, O., Onu, O., Okoro, G., Uguru, M. (2018): Overview of Biological Methods of Weed Control. – In: *Biological Approaches for Controlling Weeds*. InTech, London. <https://doi.org/10.5772/intechopen.76219>.
- [5] Bailey, K. L., Pitt, W. M., Falk, S., Derby, J. (2011): The effects of *Phoma macrostoma* on nontarget plant and target weed species under greenhouse and field conditions. – *Biological Control* 58(3): 379-386.
- [6] Bale, J. S., Van Lenteren, J. C., Bigler, F. (2008): Biological control and sustainable food production. – *Philosophical Transactions of the Royal Society B: Biological Sciences* 363(1492): 761-776.
- [7] Bergman Lodin, J., Paulson, S., Mugenyi, M. S. (2012): New seeds, gender norms and labor dynamics in Hoima District, Uganda. – *Journal of Eastern African Studies* 6: 405-422.
- [8] Blin, K., Shaw, S., Kloosterman, A. M., Charlop- Z., Powers, van Wezel, G. P., Medema, M. H. (2021): AntiSMASH 6.0: improving cluster detection and comparison capabilities. – *Nucleic Acids Research* 49: gkab335.
- [9] Bordin, E. R., Camargo, A. F., Stefanski, F. S., Scapini, T., Bonatto, C., Zanivan, J., Preczeski, K., Modkovski, T., Junior, F., Mossi, A. (2021): Current production of bioherbicides: mechanisms of action and technical and scientific challenges to improve food and environmental security. – *Biocatalysis and Biotransformation* 39: 346-359.
- [10] Boyetchko, S. M., Roskopf, E. N., Caesar, A. J., Charudattan, R. (2002): Biological weed control with pathogens: search for candidates for applications. – *Applied Mycology and Biotechnology* 2: 239-274. [https://doi.org/10.1016/S1874-5334\(02\)80013-2](https://doi.org/10.1016/S1874-5334(02)80013-2).
- [11] Boyette, C. D., Hoagland, R. E., Weaver, M. A. (2002): Interaction of a Bioherbicide and Glyphosate for Controlling Kudzu (*Pueraria lobata*). – *Biocontrol Science and Technology* 12(2): 161-168.
- [12] Byer, K., Peng, G., Wolf, T., Caldwell, B. (2006): Spray retention and its effect on weed control by mycoherbicides. – *Biological Control: Theory and Application in Pest Management* 37(3): 307-313.

- [13] Caesar, A., Caesar, T., Maathuis, M. (2010): Pathogenicity, characterization and comparative virulence of *Rhizoctonia* spp. from insect-galled roots of *Lepidium draba* in Europe. – Biological Control 52(2): 140-144.
- [14] Cheng, L., DiTommaso, A., Kao- J., Kniffin, (2022): Opportunities for microbiome suppression of weeds using regenerative agricultural technologies. – Front. Soil Sci. 2:838595. doi: 10.3389/fsoil.2022.838595.
- [15] Cimmino, A., Masi, M., Evidente, M., Superchi, S., Evidente, A. (2015): Fungal phytotoxins with potential herbicidal activity: chemical and biological characterization. – Natural Products Reports 32: 1629-1653.
- [16] Cliquet, Zeeshan, S. K. (2008): Impact of nutritional conditions on yields, germination rate and shelf-life of *Plectosporium alismatis* conidia and chlamydospores as potential candidates for the development of a mycoherbicide of weeds in rice crops. – Biocontrol Science and Technology 18(7-8): 685-695.
- [17] Cordeau, S., Triolet, M., Wayman, S., Steinberg, C., Guillemain, J. (2016): Bioherbicides: dead in the water? A review of the existing products for integrated weed management. – Crop Protection 87: 44-49.
- [18] Dahiya, A., Chahar, K., Sindhu, S. S. (2019): The rhizosphere microbiome and biological control of weeds: a review. – Spanish Journal of Agricultural Research 17(4): e10R01.
- [19] Delye, C., Jasieniuk, M., Le Corre, V. (2013): Deciphering the evolution of herbicide resistance in weeds. – Trends in Genetics 29(11): 649-658.
- [20] Gianessi, L. P., (2013): The increasing importance of herbicides in worldwide crop production. – Pest Management Science 69: 1099-1105.
- [21] Gianessi, L. P., Reigner, N. P. (2007): The value of herbicides in U.S. crop production. – Weed Technology 21(2): 559-566. <https://doi.org/10.1614/WT-06-130.1>.
- [22] Hasan, M., Ahmad- M. S., Hamdani, Rosli, A. M., Hamdan, H. (2021): Bioherbicides: an eco-friendly tool for sustainable weed management. – Plants 10: 1212.
- [23] Heap, I., (2015): The international survey of herbicide resistant weeds. – www.weedscience.org.
- [24] Hoagland, R. E., (2001): Microbial allelochemicals and pathogens as bioherbicidal agents. – Weed Technology 15: 835-857.
- [25] Hussain, M. I., Araniti, F., Schulz, M., Baerson, S., Vieites-Álvarez, Y., Rempelos, L., Bilsborrow, P., Chinchilla, N., Macías, F. A., Weston, L. A., Reigosa, M. J., Sánchez-Moreiras, A. M. (2022): Benzoxazinoids in wheat allelopathy: from discovery to application for sustainable weed management. – Environmental and Experimental Botany 202: 104997.
- [26] Inderjit, Weston, L. A., Duke, S. O. (2005): Challenges, achievements, and opportunities in allelopathy research. – Journal of Plant Interactions 1: 69-81.
- [27] Jilani, G., Mahmood, S., Chaudhry, A. N., Hassan, I., Akram, M. (2008): Allelochemicals: sources, toxicity and microbial transformation in soil—a review. – Annals of Microbiology 58: 351-357.
- [28] Jinnah, H. A., Stewart- S. M., Wade, Boland, G. J. (2010): Factors affecting the efficacy of *Pseudomonas fluorescens* Strain CL145A as a microbial herbicide for Eurasian watermilfoil (*Myriophyllum spicatum*). – Journal of Aquatic Plant Management 48(1): 49-56.
- [29] Katz, L., Baltz, R. H. (2016): Natural product discovery: past, present, and future. – Journal of Industrial Microbiology and Biotechnology 43: 5.
- [30] Kaur, S., Kaur, R., Bhagirath, S., and Chauhan, (2018): Understanding crop-weed-fertilizer-water interactions and their implications for weed management in agricultural systems. – Crop Protection 103: 65-72.
- [31] Kennedy, A. C., Stubbs, T. L. (2007): Management effects on the incidence of jointed goat grass inhibitory rhizobacteria. – Biological Control 40(2): 213-221.
- [32] Kennedy, A. C., Johnson, B. N., Stubbs, T. L. (2001): Host range of a deleterious rhizobacterium for biological control of downy brome. – Weed Science 49: 792-797.

- [33] Kong, C. H., Xuan, T. D., Khanh, T. D., Tran, H. D., Trung, N. T. (2019): Allelochemicals and signaling chemicals in plants. – *Molecules* 24(15): 2737.
- [34] Kostina-Bednarz, M., Płonka, J., Barchanska, H. (2023): Allelopathy as a source of bioherbicides: challenges and prospects for sustainable agriculture. – *Reviews in Environmental Science and Biotechnology* 22: 471-504.
- [35] Kremer, R. J., (2005): Bioherbicides in weed management. – *Biopesticides International* 1(3-4): 127-141.
- [36] Kremer, R. J., Kennedy, A. C. (1996): Rhizobacteria as biocontrol agents of weeds. – *Weed Technology* 10: 601-609.
- [37] Kubiak, A., Wolna- A., Maruwka, A. Niewiadomska, Pilarska, A. A. (2022): The Problem of weed infestation of agricultural plantations vs. the assumptions of the European biodiversity strategy. – *Agronomy* 12(8): 1808.
- [38] Kumar, A., Agarwal, S. (2024): Exploring principles and protocols for biological weed control: a comprehensive review. – *International Journal of Advanced Biochemistry Research* 8(2): 301-306.
- [39] Lareen, A., Burton, F., Schafer, P. (2016): Plant root-microbe communication in shaping root microbiomes. – *Plant Molecular Biology* 90(6): 575-587.
- [40] Lawrie, J., Greaves, M., Down, V., Western, N. (2002): Studies of spray application of microbial herbicides in relation to conidial propagule content of spray droplets and retention on target. – *Biocontrol Science and Technology* 12(1): 107-119.
- [41] Lazarus, B. E., Feris, K., Germino, M. J. (2021): Weed-suppressive bacteria effects differ in culture compared to in soils and with or without microbial competition and separation of active ingredient. – *Biological Control* 152: 104422. DOI: 10.1016/j.biocontrol.2020.104422.
- [42] Li, Y., Sun, Z., Zhuang, X., Xu, L., Chen, S., Li, M. (2003): Research progress on microbial herbicides. – *Crop Protection* 22(2): 247-252.
- [43] Macias, F. A., Molinillo, J. M. G., Varela, R. M., Galindo, J. C. G. (2007): Allelopathy: a natural alternative for weed control. – *Pest Management Science* 63(4): 327-348.
- [44] Mejri, D., Gamalero, E., Tombolini, R., Musso, C., Massa, N., Berta, G., Souissi, T. (2010): Biological control of great brome (*Bromus diandrus*) in durum wheat (*Triticum durum*): specificity, physiological traits and impact on plant growth and root architecture of the fluorescent pseudomonad strain X33d. – *Biocontrol* 55: 561-572.
- [45] Mohanram, S., Kumar, P. (2019): Rhizosphere microbiome: revisiting the synergy of plant-microbe interactions. – *Annals of Microbiology* 69: 307-320.
- [46] Mushtaq, W., Siddiqui, M. B., Hakeem, K. R. (2020): History of Allelopathy. – In: *Allelopathy. SpringerBriefs in Agriculture*. Cham, Springer. pp. 1-12. <https://doi.org/10.1007/978-3-030-40807-7>.
- [47] Mynavathi, V. S., Prabhakaran, N. K., Chinnusamy, C. (2015): Manually-operated weeders for time saving and weed control in irrigated maize. – *Indian Journal of Weed Science* 47(1): 98-100.
- [48] Nisa, H., Kamili, A. N., Nawchoo, I. A., Shafi, S., Shameem, N., Bandh, S. A. (2015): Fungal Endophytes as prolific source of phytochemicals and other bioactive natural products: a review. – *Microbial Pathogenesis* 82: 50-59.
- [49] Ogwuiké, P., Rodenburg, J., Diagne, A., Agboh- A., Noameshie, E. Amovin-Assagba, (2014): Weed management in upland rice in sub-Saharan Africa: impact on labour and crop productivity. – *Food Security* 6: 327-337. <https://doi.org/10.1007/s12571-014-0351-7>.
- [50] Pacanoski, Z., (2015): Bioherbicides. – In: Price, A. J., Kelton, J. A. (eds.) *Herbicides, Physiology of Action, and Safety*. IntechOpen, London. pp. 1-12. <https://doi.org/10.5772/61528>.
- [51] Perotti, V. E., Larran, S., A., S., Palmieri, V. E., Martinatto, A. K., Permingeat, H. R. (2020): Herbicide resistant weeds: a call to integrate conventional agricultural practices, molecular biology knowledge and new technologies. – *Plant Science* 290: 110255.

- [52] Phour, M., Sindhu, S. S. (2019): Bioherbicidal effect of 5-aminolevulinic acid producing rhizobacteria in suppression of *Lathyrus aphaca* weed growth. – *Biocontrol* 64: 221-232.
- [53] Phukan, J., Deka, J., Kurmi, K., Kalita, S. (2021): Deleterious Rhizobacteria as a potential bioherbicide—a review. – *International Journal of Agriculture and Environmental Sciences* 8: 1-5.
- [54] Powles, S. B., Yu, Q. (2010): Evolution in action: plants resistant to herbicides. – *Annual Review of Plant Biology* 61: 317-347.
- [55] Radhakrishnan, R., Alqarawi, A. A., Abd Allah, E. F. (2018): Bioherbicides: current knowledge on weed control mechanism. – *Ecotoxicology and Environmental Safety* 158: 131-138.
- [56] Ramesh, K., Matloob, A., Aslam, F., Florentine, S., Chauhan, B. S. (2017): Weeds in a changing climate: vulnerabilities, consequences, and implications for future weed management. – *Frontiers in Plant Science* 8: 95. <https://doi.org/10.3389/fpls.2017.00095>.
- [57] Rayamajhi, M., Pratt, P., Center, T., Van, T. (2010): Insects and a pathogen suppress *Melaleuca quinquenervia* cut-stump regrowth in Florida. – *Biological Control* 53(1): 1-8.
- [58] Reddy, K. N., Nandula, V. K. (2012): Herbicide resistant crops: history, development and current technologies. – *Indian Journal of Agronomy* 57(1): 1-7.
- [59] Reiss, A., Fomsgaard, I. S., Mathiassen, S. K., Kudsk, P. (2018): Weed suppressive traits of winter cereals: allelopathy and competition. – *Biochemical Systematics and Ecology* 76: 35-41.
- [60] Sehrawat, A., Sindhu, S. S. (2019): Potential of biocontrol agents in plant disease control for improving food safety. – *Defence Life Science Journal* 4: 220-225.
- [61] Sindhu, S. S., Sehrawat, A., Sharma, R., Dahiya, A., Khandelwal, A. (2017): Belowground Microbial Crosstalk and Rhizosphere Biology. – In: Singh, D. P. et al. (eds.) *Plant-Microbe Interactions in Agro-Ecological Perspectives*. Springer Nature, Singapore, pp. 695-752.
- [62] Sindhu, S. S., Khandelwal, A., Phour, M., Sehrawat, A. (2018): Bioherbicidal Potential of Rhizosphere Microorganisms for Ecofriendly Weed Management. – In: Meena, V. S. (ed.) *Role of Rhizospheric Microbes in Soil*. Springer Nature, Singapore, pp. 331-376.
- [63] Singh, R., Kumar, V., Sharma, P. (2017): Comparative labor requirements for weed control in small-scale and large-scale farming systems. – *Agronomy Journal* 109(3): 595-604. <https://doi.org/10.2134/agronj2016.10.0584>.
- [64] Skinnider, M. A., Merwin, N. J., Johnston, C. W., Magarvey, N. A. (2017): PRISM 3: Expanded prediction of natural product chemical structures from microbial genomes. – *Nucleic Acids Research* 45: gkx320.
- [65] Soltys, D., Krasuska, U., Bogatek, R., Gniazdowski, A. (2013): Allelochemicals as Bioherbicides—Present and Perspectives. – In: Price, A. J., Kelton, J. A. (eds.) *Herbicides: Current Research and Case Studies in Use*. InTech, London. <https://doi.org/10.5772/56185>.
- [66] Sondhia, S., Singh, P. K. (2018): Bioefficacy and fate of pendimethalin residues in soil and mature plants in chickpea field. – *Journal of Research on Weed Science* 1(2018): 28-39.
- [67] Stokes, J. M., Yang, K., Swanson, K., Jin, W., Cubillos- A., Ruiz, Donghia, N. M. (2020): A deep learning approach to antibiotic discovery. – *Cell* 180: 688-702.
- [68] Stubbs, T., Kennedy, A. C. (2012): Microbial Weed Control and Microbial Herbicides. – In: Alvarez-Fernandez. R. (ed.) *Herbicides: Environmental Impact Studies and Management Approaches*. InTech, London.
- [69] Swanton, C. J., Nkoa, R., Blackshaw, R. E. (2015): Experimental methods for crop–weed competition studies. – *Weed Science* 63: 2-11. <https://doi.org/10.1614/WS-D-13-00062.1>.
- [70] Trognitz, F., Hackl, E., Widhalm, S., Sessitsch, A. (2016): The role of plant–microbiome interactions in weed establishment and control. – *FEMS Microbiology Ecology* 92(10): 138.

- [71] Ward, S., Reid, S., Harrington, J., Sutton, J., Beck, K. (2008): Genetic variation in invasive populations of yellow toadflax (*Linaria vulgaris*) in the Western United States. – Weed Science 56(3): 394-399.
- [72] Xiao, Z., Zou, T., Lu, S., Xu, Z. (2020): Soil microorganisms interacting with residue-derived allelochemicals effects on seed germination. – Saudi Journal of Biological Sciences 27(4): 1057-1065.
- [73] Xu, D., Xue, M., Shen, Z., Jia, X., Hou, X., Lai, D., Zhou, L. (2021): Phytotoxic Secondary Metabolites from Fungi. – Toxins 13(4): 261.
- [74] Zeller, S. L., Brand, H., Schmid, B. (2007): Host-plant selectivity of Rhizobacteria in a crop/weed model system. – PLoS ONE 2: 846-854.