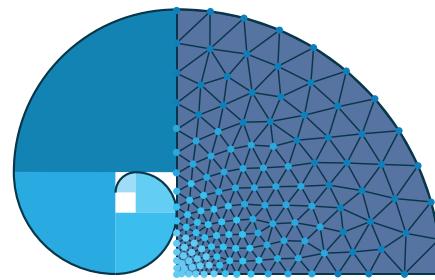


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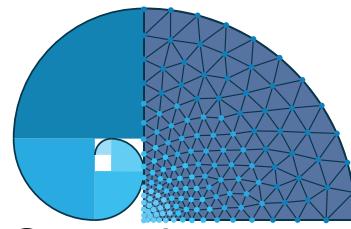
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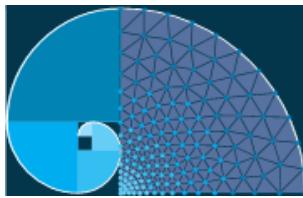
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A Decision-Ready Framework for Sustainable Land-Use Planning Using Design Science Research

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Abstract

Sustainable land-use planning involves complex decision-making under multiple, often conflicting objectives, regulatory constraints, and uncertainty. Conventional planning approaches frequently rely on fragmented analyses or case-specific tools that limit transparency, reusability, and defensibility of decisions. To address these limitations, this study applies a Design Science Research (DSR) approach to design and evaluate a decision support framework for sustainable land-use planning. The proposed framework is conceptualized as a reusable decision support artifact that structures land-use decisions through explicit sustainability criteria, feasibility constraints, and uncertainty analysis. It integrates environmental, economic, social, and governance dimensions within a transparent decision workflow, enabling systematic comparison of competing land-use alternatives. In line with DSR principles, the contribution of this study lies in the design of a generalizable framework and associated design knowledge, rather than in the implementation of a specific software system. The framework is demonstrated using a protected-area land-use planning scenario informed by technoenvironmental exploration practices in the Bombo-Lumene Hunting Estate and Reserve in the Democratic Republic of Congo. Evaluation focuses on utility, transparency, and robustness of decision outcomes. Sensitivity analysis is employed to examine the stability and interpretability of alternative rankings under varying planning priorities, complemented by qualitative assessment of decision traceability. The results indicate that the framework supports structured and defensible land-use decision-making by making sustainability trade-offs explicit and by enhancing robustness to changing assumptions. Overall, the study demonstrates the applicability of DSR to spatial planning and sustainability challenges and contributes a decision-ready framework adaptable to diverse land-use planning contexts worldwide.

1. Introduction

Land-use planning is a key instrument for achieving sustainable development, as it governs how land is allocated among competing environmental, economic, and social objectives. Decisions related to zoning, activity allocation, and development intensity involve complex

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trade-offs, spatial constraints, and long-term consequences. These challenges are intensified by rapid urbanization, environmental degradation, and increasing regulatory pressures, making land-use planning an inherently complex and uncertain decision-making problem.

Recent studies highlight the growing use of decision support approaches to address this complexity. GIS-based multi-criteria analysis has been applied to evaluate land suitability and sustainability trade-offs in developing regions (Thapa et al., 2025). Scenario-based planning frameworks enable planners to explore alternative land-use futures under uncertainty and compare development pathways (Sedighi et al., 2025). In parallel, systematic reviews of digital land-use decision support tools report increasing adoption in planning practice while identifying persistent challenges related to transparency, integration, and usability (Poggi et al., 2025). Together, these findings indicate a clear demand for structured and interpretable decision support in land-use planning.

Despite these advances, land-use planning practice often relies on fragmented or ad hoc approaches. Decisions are frequently informed by expert judgment, isolated spatial analyses, or single-criterion assessments that do not systematically integrate sustainability dimensions or make assumptions explicit. Although analytical and spatial tools are widely available, they are not always embedded within coherent decision frameworks that support comparison of alternatives, stakeholder engagement, and robustness analysis. As a result, planning outcomes may be difficult to justify, reproduce, or adapt when priorities or boundary conditions change.

Decision support frameworks have been proposed to address these limitations by structuring complex planning problems and integrating heterogeneous criteria. However, many existing approaches remain highly context-specific or focus primarily on technical optimization, limiting their generalizability and interpretability. In particular, there is a lack of reusable decision support frameworks explicitly designed for sustainable land-use planning across diverse institutional and geographic contexts, while accommodating regulatory constraints and uncertainty.

Design Science Research (DSR) provides a suitable methodological foundation for addressing this gap. DSR emphasizes the purposeful design and evaluation of artifacts—such as models, methods, and frameworks—that address identified real-world problems (Hevner et al., 2004). Conceptual frameworks are recognized as legitimate DSR artifacts when they encapsulate transferable design knowledge applicable to a class of problems rather than a single implementation (Gregor & Hevner, 2013). This perspective aligns well with land-use planning, where decision support must balance rigor with flexibility, transparency, and interpretability. Figure 1 provides a conceptual overview of the DSR framework adopted in this study and its integration with sustainability dimensions in land-use planning.

Recent methodological guidance further emphasizes that DSR artifacts may be demonstrated and evaluated through realistic scenarios rather than full operational deployment, particularly in cross-disciplinary domains such as spatial planning and sustainability (Delport et al., 2024). This allows evaluation of decision logic, robustness, and explanatory value—qualities that are central to effective land-use planning but often underemphasized in optimization-driven approaches. Akoka et al. (2023) analyze how knowledge is dynamically created in DSR projects by identifying paths of knowledge contributions, deriving seven DSR strategies, and providing guidelines to support the initiation and progression of DSR studies.

Against this background, the objective of this study is to design and evaluate a decision support framework for sustainable land-use planning using a DSR approach. The proposed framework integrates multi-dimensional sustainability criteria, feasibility constraints, and uncertainty considerations into a systematic and well-structured decision-making process. Rather than prescribing a single optimal solution, it supports informed interpretation of trade-offs among land-use alternatives.

The contribution of this paper is twofold. First, it provides a generalizable decision support artifact tailored to sustainable land-use planning challenges. Second, it demonstrates how DSR can be effectively applied to spatial planning problems through abstraction and scenario-based evaluation. The paper is structured as follows: Section 2 reviews related work, Section 3 presents the research methodology, Section 4 describes the decision support artifact, Section 5 demonstrates and evaluates the artifact using a protected-area planning context, Section 6 discusses the findings, and Section 7 concludes the paper.

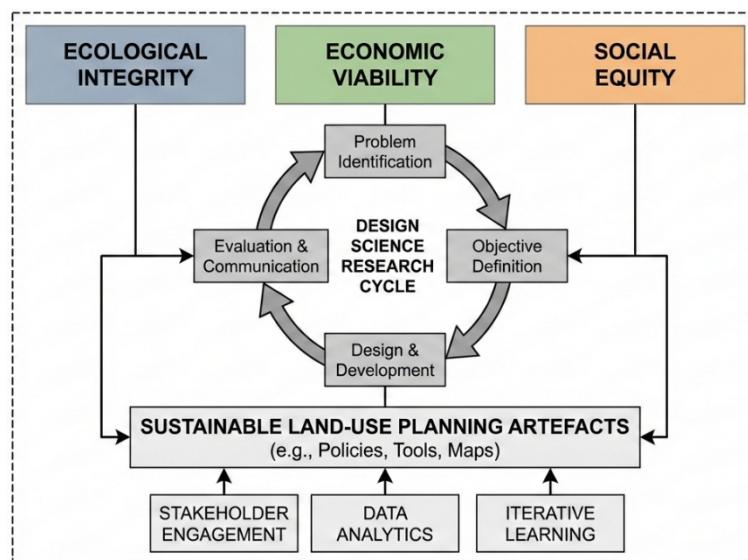


Figure 1. DSR framework integrating sustainability dimensions into land-use planning

2. Related Work

This study builds on a body of literature that addresses DSR from theoretical, methodological, evaluative, and applied perspectives, as well as domain-specific studies that motivate the need for decision support in land-use planning. To provide a coherent synthesis and avoid fragmented citation, the related literature is structured into four main streams.

2.1. Foundational DSR theory

The first stream establishes the theoretical foundations of DSR and defines its core concepts. Seminal work by Hevner et al. (2004) positions DSR as a problem-solving research paradigm focused on the purposeful design and evaluation of artifacts that address real-world problems. Within this paradigm, artifacts may take the form of constructs, models, methods, or frameworks, and their value lies in utility, rigor, and relevance.

Building on this foundation, Gregor and Hevner (2013) further clarify how DSR contributions should be positioned and communicated. They emphasize that design knowledge embodied in conceptual models and frameworks constitutes a legitimate and impactful research contribution, even in the absence of a fully instantiated software system. This perspective is particularly relevant for studies that aim to generalize decision logic across a class of problems rather than optimize a single technical solution.

Together, these foundational works provide the theoretical basis for treating a decision support framework for sustainable land-use planning as a valid DSR artifact and for evaluating its contribution in terms of transparency, robustness, and reusability.

2.2. DSR methodologies and research design guidance

The second stream focuses on methodological guidance for conducting DSR studies. Peffers et al. (2007) propose a widely adopted DSR Methodology that structures DSR into activities such as problem identification, objective definition, artifact design, demonstration, and evaluation. This process-oriented view has influenced numerous DSR applications across domains. March and Storey (2008) introduce DSR in the information systems discipline by outlining its foundations, significance, and role in addressing real-world problems, thereby framing the contributions of the special issue on DSR.

More recent contributions emphasize flexibility and methodological choice in DSR. Venable et al. (2017) discusses how researchers can select appropriate DSR methodologies depending on problem context, artifact type, and evaluation goals. Delport et al. (2024) provide updated methodological guidelines that highlight common challenges faced by DSR researchers, particularly when designing conceptual artifacts or working in cross-disciplinary settings. They stress the importance of abstraction, clarity of design requirements, and alignment between artifact purpose and evaluation strategy. Recent syntheses of DSR further consolidate these foundational principles and emphasize the role of abstraction and generalization in artifact design (vom Brocke et al., 2020).

The DSR Grid proposed by vom Brocke and Maedche (2019) further supports systematic planning of DSR studies by mapping design choices across multiple dimensions, such as problem relevance, artifact scope, and evaluation depth. In addition, Thuan et al. (2019) focus on the construction of research questions in DSR, emphasizing the distinction between design-oriented questions and explanatory questions.

These methodological contributions collectively inform the research design adopted in this study, particularly the choice to develop an implementation-independent framework, demonstrate it using a realistic planning context, and evaluate it through robustness and interpretability rather than technical performance alone.

2.3. Evaluation, knowledge production, and quality in DSR

The third stream addresses how DSR artifacts are evaluated and how they contribute to knowledge. Venable et al. (2016) propose the Framework for Evaluation in Design Science (FEDS), which emphasizes that evaluation strategies should be aligned with artifact maturity, purpose, and context. Rather than prescribing a single evaluation method, FEDS supports a range of formative and summative evaluations, including analytical, experimental, and qualitative approaches.

Baskerville et al. (2015) further enrich this perspective by introducing the concept of multiple genres of inquiry in DSR. They argue that a single DSR study may legitimately combine design, evaluation, and reflection, and that knowledge can be generated not only through empirical testing but also through structured reasoning and interpretive analysis.

These perspectives support the evaluation approach adopted in this study, where the emphasis is placed on decision transparency, robustness under uncertainty, and practical interpretability, rather than on predictive accuracy or system performance metrics.

2.4. Applied DSR and domain-oriented planning studies

The fourth stream includes applied and hybrid DSR studies, as well as domain-specific literature that motivates the research problem. Action design research, introduced by Sein et al. (2011), demonstrates how DSR can be conducted in close interaction with real-world contexts while maintaining theoretical rigor. Other applied studies, such as Apiola and Sutinen (2020), Castro et al. (2025), Teixeira et al. (2019), and Venkatesh et al. (2017), illustrate how DSR artifacts can take the form of decision aids, service designs, or assistance systems evaluated through practical use and stakeholder engagement.

In addition to applied DSR studies, domain-oriented planning research provides the problem context for this study. In particular, techno-environmental planning research conducted in protected areas, such as the Bombo-Lumene Hunting Estate and Reserve in the Democratic Republic of Congo (Monkenya et al., 2025), highlights the land-use nature of decision-making under environmental and regulatory constraints. These studies demonstrate that planning choices related to activity allocation, spatial configuration, and method selection are fundamentally land-use decisions requiring structured and transparent decision support.

2.5. Research gap

The reviewed literature indicates that while DSR provides a robust theoretical and methodological foundation for artifact-based research, and while land-use planning challenges are well documented in applied studies, there remains a gap in the form of generalizable decision support frameworks explicitly designed for sustainable land-use planning using a DSR approach. Existing planning studies often rely on case-specific analyses, while many DSR studies do not address spatial sustainability problems. This study addresses this gap by designing and evaluating a decision support framework that integrates sustainability criteria, constraints, and uncertainty within a DSR paradigm.

3. Research Methodology

3.1. DSR approach

DSR focuses on the purposeful creation of artifacts—such as models, methods, and frameworks—that address real-world problems, with scientific knowledge generated through the design and evaluation of these artifacts (Delport et al., 2024). This study adopts a DSR methodology to design and evaluate a decision support framework for sustainable land-use planning.

DSR is well suited to this research because it emphasizes both practical relevance and methodological rigor, making it appropriate for complex decision-making contexts characterized by multiple criteria, uncertainty, and regulatory constraints. Unlike descriptive or explanatory research approaches, DSR explicitly aims to produce actionable solutions while contributing transferable design knowledge. Following established DSR guidance, the contribution of this study is positioned as design knowledge embodied in a decision support framework, rather than as a fully instantiated software system (Gregor & Hevner, 2013).

The DSR process adopted in this study follows an iterative progression from problem identification and objective definition to artifact design, demonstration, and evaluation. This process is illustrated in Figure 2.



Figure 2. Iterative DSR process guiding artifact development and evaluation

3.2. Problem identification and design objectives

The research is motivated by the absence of systematic and reusable frameworks that support sustainable land-use decisions involving multiple criteria and uncertainty. As discussed in Section 1, existing land-use planning practices often rely on fragmented analyses that limit comparability, traceability, and robustness of decisions.

Based on this problem, the following design objectives were defined:

- support systematic evaluation of land-use alternatives across multiple sustainability dimensions;
- enable transparent and traceable decision logic;
- incorporate spatial and regulatory constraints into the decision process;
- address uncertainty and sensitivity to stakeholder preferences;
- allow adaptation to different land-use planning contexts.

These objectives guided the design and development of the proposed artifact.

3.3. Artifact design and development

The primary artifact of this research is a decision support framework for sustainable land-use planning. The framework was designed as a modular structure comprising a decision workflow, a multi-criteria evaluation model, and an uncertainty analysis component. Concepts from land-use planning, multi-criteria decision analysis, and sustainability assessment were employed as kernel theories to inform the design and ensure methodological rigor.

The artifact is implementation-independent, allowing it to be applied using different technical platforms, such as spreadsheets, GIS-based environments, or web-based decision support tools. This design choice supports generalizability and reuse across diverse planning contexts.

3.4. Demonstration and evaluation

The framework is demonstrated using a representative land-use planning scenario involving competing land-use alternatives. Evaluation focuses on assessing the framework's ability to produce transparent, consistent, and robust decision outcomes. Quantitative evaluation includes sensitivity analysis to examine the stability of alternative rankings under varying criteria weights, while qualitative evaluation considers the interpretability and practical usefulness of the framework for decision-makers.

The demonstration scenario is informed by real-world protected-area planning contexts, particularly techno-environmental exploration planning studies conducted in the Bombo-Lumene Hunting Estate and Reserve. These studies illustrate the land-use nature of decision-making under strict environmental and regulatory constraints and provide a realistic context for demonstrating the proposed framework.

3.5. Research contribution

In accordance with DSR principles, this study contributes both a validated decision support artifact and design knowledge in the form of explicit design objectives, architectural choices, and evaluation insights. The adopted methodology ensures that the proposed framework is not only practically relevant but also scientifically grounded and transferable to other sustainable land-use planning contexts.

4. Design of the Decision Support Artifact

4.1. Design requirements

The primary artifact developed in this study is a Decision Support Framework for Sustainable Land-Use Planning. In DSR terms, the artifact represents a conceptual model and decision-making method that structures complex land-use problems into a transparent, systematic, and reusable decision process. The artifact is designed to be implementation-independent, allowing its application across different geographic, institutional, and technological contexts.

Following methodological guidelines for DSR, the proposed decision support framework is positioned as a conceptual artifact that is intended to be applicable to a class of land-use planning problems rather than a single site-specific implementation (Delport et al., 2024).

The purpose of the artifact is to support land-use planners and decision-makers in evaluating alternative land-use options by explicitly integrating sustainability criteria, stakeholder preferences, spatial feasibility, and uncertainty considerations. Rather than optimizing a single objective, the artifact facilitates informed decision-making by making trade-offs among competing land-use objectives explicit and traceable.

The design of the artifact was guided by the following design requirements:

1. Multi-dimensional sustainability integration: The artifact must support the simultaneous evaluation of environmental, economic, social, and governance considerations.
2. Transparency and traceability: All assumptions, criteria weights, and evaluation steps must be explicit and open to scrutiny.
3. Feasibility awareness: Spatial and regulatory constraints must be incorporated to ensure that only permissible land-use alternatives are evaluated.
4. Robustness under uncertainty: The artifact must enable assessment of how sensitive decision outcomes are to changes in preferences and assumptions.
5. Generalizability and adaptability: The artifact must be reusable across different land-use planning contexts without reliance on case-specific parameters.

These requirements form the foundation of the artifact's design and structure.

4.2. Framework architecture

The decision support artifact is organized into an integrated architecture consisting of three interrelated components: a decision workflow, a multi-criteria evaluation model, and an uncertainty assessment mechanism. The overall architecture of the proposed artifact and the interaction among these components are illustrated in Figure 3.

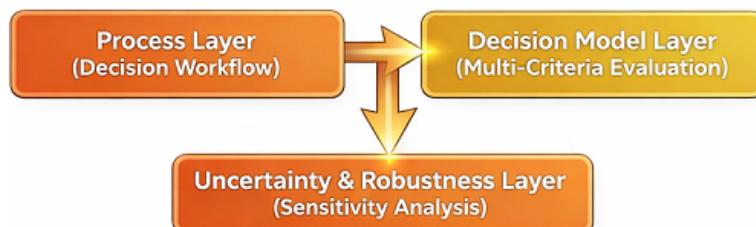


Figure 3. Architecture of the decision support artifact for sustainable land-use planning

The decision workflow provides a structured sequence of steps guiding the land-use planning process. It begins with problem definition and identification of planning objectives, followed by the specification of feasible land-use alternatives. Criteria selection and stakeholder preference elicitation are then conducted, after which alternatives are evaluated and ranked. A feedback mechanism allows iterative refinement of assumptions and preferences, supporting adaptive decision-making.

The multi-criteria evaluation model operationalizes the comparison of land-use alternatives. Using a multi-criteria decision analysis approach, the model aggregates heterogeneous criteria by combining performance scores with stakeholder-defined weights. Spatial and regulatory constraints, such as protected areas or zoning rules, are applied as feasibility filters prior to evaluation to ensure compliance with planning regulations.

In addition to supporting systematic decision-making, the proposed framework is compatible with participatory land-use planning practices, as it allows the integration of stakeholder preferences, expert judgment, and local knowledge into the evaluation of alternative land-use scenarios.

The uncertainty assessment mechanism examines the stability of decision outcomes under varying assumptions. By assessing how changes in criteria weights or input values influence alternative rankings, the artifact enables decision-makers to evaluate the robustness of planning outcomes and identify criteria that exert dominant influence on decisions.

4.3. Sustainability criteria for land-use planning

To ensure broad applicability, the artifact employs a generic sustainability criteria structure that can be adapted to different land-use contexts. The criteria are organized into four main dimensions.

- **Environmental criteria** address potential impacts on ecosystems, land cover, soil stability, and exposure to environmental risks.
- **Economic criteria** capture development costs, economic benefits, and accessibility to existing infrastructure.
- **Social criteria** reflect population needs, equity considerations, and community acceptance of land-use options.
- **Governance criteria** ensure compliance with regulatory frameworks and alignment with spatial planning policies.

This criteria structure provides a balanced representation of sustainability objectives while allowing planners to tailor specific indicators to local priorities and data availability.

4.4. Decision output and interpretation

The artifact generates a ranked set of land-use alternatives accompanied by explanatory outputs that detail the contribution of individual criteria and the results of sensitivity analysis. Rather than prescribing a single optimal solution, the framework supports decision interpretation by highlighting trade-offs among alternatives and clarifying how different sustainability priorities influence outcomes.

This emphasis on interpretability and transparency aligns the artifact with the normative and participatory nature of land-use planning. By making decision logic explicit, the artifact facilitates communication among planners, policymakers, and stakeholders and supports informed, defensible land-use decisions.

5. Demonstration and Evaluation

5.1. Demonstration scenario: Protected-area land-use planning context

The proposed decision support artifact was demonstrated using a protected-area land-use planning scenario informed by techno-environmental exploration planning practices in the Bombo-Lumene Hunting Estate and Reserve in the Democratic Republic of Congo. This

reserve represents a highly constrained land-use environment in which economic activities are permitted only if they comply with strict environmental, spatial, and regulatory requirements, and where geospatial and remote sensing techniques have been used to support ecosystem monitoring and land-use control (Kalambay et al., 2024). In accordance with DSR guidelines, the artifact is demonstrated through a realistic planning context rather than full operational deployment, allowing evaluation of its decision logic, transparency, and robustness.

In the Bombo-Lumene context, land-use decisions require balancing conservation objectives with development-oriented activities, such as geological and geophysical exploration, while minimizing impacts on biodiversity and local communities. Prior studies emphasize planning choices such as prioritizing non-invasive airborne surveys, carefully orienting survey lines, and avoiding disruptive exploration techniques as effective mechanisms for reducing environmental disturbance. These choices are inherently land-use decisions, as they determine how, where, and under what conditions activities are permitted within a protected area.

For the purposes of this study, the Bombo-Lumene planning context was abstracted into a generic land-use decision problem involving competing land-use alternatives, including restricted-use zones, conservation-oriented areas, and controlled development activities. This abstraction enables demonstration of the decision support framework without reliance on site-specific operational data, while preserving the essential characteristics of protected-area land-use planning under environmental and regulatory constraints.

The relationship between the protected-area planning context, land-use alternatives, and the decision support artifact is illustrated in Figure 4.

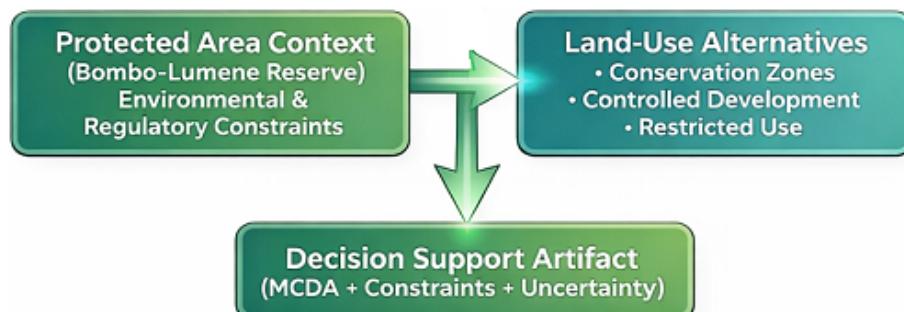


Figure 4. Demonstration context of the decision support artifact for sustainable land-use planning

5.2. Application of the decision support artifact

The demonstration followed the workflow defined by the proposed decision support artifact. First, the planning problem was specified in terms of sustainability objectives, with emphasis on environmental protection, regulatory compliance, and controlled development. Land-use alternatives were then defined to represent distinct planning strategies consistent with the constraints of a protected-area context.

Sustainability criteria were applied across environmental, economic, social, and governance dimensions, reflecting key considerations identified in techno-environmental planning studies of the Bombo-Lumene reserve. The selected criteria and their qualitative descriptions are summarized in Table 1. Criteria weights were assigned to represent a conservation-prioritized planning perspective, consistent with protected-area management objectives; the resulting weighting scheme is reported in Table 2. Spatial and regulatory constraints were incorporated as feasibility conditions within the framework, ensuring that land-use alternatives violating protection rules were excluded from further evaluation.

Table 1. Sustainability criteria used for evaluating land-use alternatives

Criterion ID	Sustainability Dimension	Criterion Name	Description	Measurement Scale	Data Type
C1	Environmental	Biodiversity Impact	Expected impact on habitats and species diversity	Qualitative (Low-High)	Expert judgment
C2	Environmental	Ecosystem Disturbance	Degree of physical disturbance to ecosystems	Qualitative (Low-High)	Expert judgment
C3	Economic	Economic Feasibility	Viability of the land-use option under constraints	Qualitative (Low-High)	Planning assessment
C4	Social	Community Impact	Expected impact on local communities and livelihoods	Qualitative (Low-High)	Stakeholder input
C5	Governance	Regulatory Compliance	Consistency with protected-area regulations	Binary / Ordinal	Regulatory rules

Table 2. Criteria weights reflecting a conservation-prioritized planning perspective

Criterion ID	Criterion Name	Sustainability Dimension	Weight	Rationale
C1	Biodiversity Impact	Environmental	0.30	Priority protection of sensitive habitats
C2	Ecosystem Disturbance	Environmental	0.25	Minimization of physical impacts
C3	Economic Feasibility	Economic	0.15	Secondary to environmental protection
C4	Community Impact	Social	0.15	Protection of local livelihoods
C5	Regulatory Compliance	Governance	0.15	Mandatory compliance requirement

Using the multi-criteria evaluation component of the framework, the remaining land-use alternatives were assessed and ranked based on their aggregated performance across the defined criteria. The resulting ranking of land-use alternatives is presented in Table 3. In addition to producing a ranking, the framework generated explanatory outputs that indicate the relative contribution of individual criteria to the overall results. These trade-off patterns are supported by transparent interpretation of how sustainability dimensions influence the final decision outcomes.

Table 3. Aggregated evaluation and ranking of land-use alternatives

Alternative ID	Land-Use Strategy	Feasible (Y/N)	Aggregated Score	Rank	Key Strengths	Key Trade-offs
A1	Conservation-Only Zone	Yes	0.82	1	Maximum ecological protection	Limited economic activity
A2	Restricted-Use Zone	Yes	0.68	2	Balanced protection and access	Moderate disturbance

A3	Controlled Development	Yes	0.55	3	Higher economic benefits	Increased environmental risk
A4	Intensive Development	No	-	Exc.	Regulatory non-compliance	-

5.3. Evaluation approach

Evaluation of the decision support artifact focused on assessing its utility, transparency, and robustness, in accordance with DSR principles. Rather than validating numerical predictions, the evaluation examined whether the artifact effectively supports structured, traceable, and defensible land-use decision-making in a protected-area context.

A sensitivity analysis was conducted by systematically varying criteria weights within plausible ranges to assess the stability of land-use alternative rankings. This analysis explored how shifts in planning priorities—such as increased emphasis on environmental protection or development feasibility—affected decision outcomes and whether ranking changes remained interpretable and consistent with planning expectations.

In addition, a qualitative evaluation was performed to assess the interpretability and practical usability of the framework. Particular attention was given to the clarity of the decision logic, the transparency and traceability of underlying assumptions, and the extent to which results could be clearly communicated to planners and stakeholders involved in protected-area management. Together, these evaluation activities provide evidence of the artifact's suitability for supporting sustainable land-use planning decisions under environmental and regulatory constraints.

5.4. Evaluation results

The evaluation results indicate that the proposed framework produces robust and interpretable decision outcomes under protected-area land-use constraints. Sensitivity analysis showed that alternative rankings remained stable across a wide range of criteria weight variations, particularly when environmental and governance criteria were prioritized, reflecting the conservation-oriented nature of protected-area planning.

When ranking changes occurred under alternative weighting scenarios, the framework clearly identified the criteria responsible for these changes, enhancing transparency and supporting informed discussion. The qualitative evaluation confirmed that the structured workflow and explicit criteria definitions improved the comprehensibility of planning decisions, compared to ad hoc or implicitly defined planning approaches commonly observed in technoenvironmental studies.

5.5. Summary of demonstration and evaluation

Overall, the demonstration confirms that the proposed decision support artifact is well suited for sustainable land-use planning in protected areas, such as the Bombo-Lumene Hunting Estate and Reserve. By abstracting real-world technoenvironmental planning practices into a generalized decision framework, the artifact enables transparent, robust, and reusable land-use decision-making. This demonstration satisfies the DSR requirement of showing the artifact's applicability in a realistic problem context while maintaining generalizability beyond a single case.

6. Discussion

This study demonstrates how a DSR approach can be used to develop a transparent and decision-ready framework for sustainable land-use planning. By structuring the decision

process around explicit sustainability criteria, feasibility constraints, stakeholder preferences, and uncertainty analysis, the proposed framework addresses key limitations of conventional land-use planning practices. In particular, it enhances decision traceability and makes sustainability trade-offs explicit, thereby supporting more informed and defensible planning outcomes.

Compared to existing land-use decision support approaches, the proposed framework emphasizes reusability and methodological clarity rather than case-specific optimization. For example, GIS-based multi-criteria analysis has been widely applied to land suitability assessment in developing regions, providing valuable spatial insights but often remaining tightly coupled to local datasets and technical configurations (Thapa et al., 2025). Similarly, spatial scenario planning frameworks support exploration of alternative land-use futures under uncertainty but typically focus on generating plausible scenarios rather than structuring a generalizable decision logic (Sedighi et al., 2025). In contrast, the framework proposed in this study abstracts from specific spatial implementations and formalizes decision reasoning in a way that can be transferred across planning contexts.

The integration of sensitivity analysis further distinguishes the framework from many existing approaches. While recent reviews highlight the growing availability of digital land-use decision support tools, they also note recurring challenges related to transparency, interpretability, and robustness of results (Poggi et al., 2025). By explicitly examining how changes in criteria weights affect decision outcomes, the proposed framework enables decision-makers to understand the implications of shifting priorities and evolving policy objectives—an essential feature in dynamic land-use contexts.

The demonstration in a protected-area planning context illustrates the value of abstraction in DSR. Previous techno-environmental planning studies addressing protected-area exploration challenges have relied primarily on site-specific analyses to minimize environmental impacts through careful selection of exploration techniques and spatial configurations. While operationally effective, such approaches are often difficult to generalize. The proposed framework builds on these insights but generalizes them into a reusable land-use decision support artifact applicable across diverse protected-area settings.

From a methodological perspective, the study reinforces the suitability of DSR for addressing spatial planning and sustainability challenges. Consistent with DSR principles, the primary contribution lies in the design of a reusable framework and associated design knowledge applicable to a class of land-use planning problems, rather than in predictive accuracy or system deployment (Gregor & Hevner, 2013).

From a practical perspective, the proposed framework offers planning agencies and policymakers a structured decision-support approach for evaluating land-use alternatives under multiple sustainability criteria and uncertainty. By enabling systematic comparison of scenarios, the framework can support evidence-informed policy development, spatial planning, and sustainability assessments. For practitioners, including urban planners and environmental managers, the framework provides a flexible tool that can be adapted to different planning contexts and data availability levels, facilitating the integration of sustainability considerations into routine planning and decision-making processes.

Several limitations should be acknowledged. The demonstration relied on an abstract planning scenario and hypothetical preference structures rather than empirical data and direct stakeholder engagement. In addition, the framework was evaluated analytically rather than through full operational implementation. These limitations reflect the scope of a short DSR study and highlight opportunities for future work, including empirical calibration, participatory evaluation, and integration with spatial decision support systems.

7. Conclusion

This study presented the design and evaluation of a decision support framework for sustainable land-use planning using a DSR approach. Addressing the complexity of land-use decisions involving multiple sustainability objectives, uncertainty, and regulatory constraints, the research responds to the need for transparent and reusable decision support tools applicable across planning contexts.

The primary contribution is an implementation-independent decision support artifact that structures land-use decision problems through explicit sustainability criteria, feasibility constraints, and uncertainty analysis. By embedding decision logic within a clear and traceable workflow, the framework supports defensible evaluation of land-use alternatives while remaining adaptable to different planning settings. The study thus contributes transferable design knowledge applicable to a class of land-use planning problems.

The framework was demonstrated using a protected-area planning scenario informed by techno-environmental practices in the Bombo-Lumene Hunting Estate and Reserve, showing its ability to support systematic comparison of competing land-use strategies. Sensitivity analysis indicated that decision outcomes remain interpretable under changing planning priorities.

Limitations include the use of an abstract scenario and the absence of full operational implementation. Future work may extend the framework through empirical calibration, stakeholder engagement, or integration with spatial decision support systems. Overall, the study illustrates the potential of DSR to advance transparent and decision-ready land-use planning.

Authorship Contribution Statement

The author is solely responsible for the conceptualization, methodology, analysis, and manuscript preparation.

Conflict of Interest

The author declares no conflict of interest.

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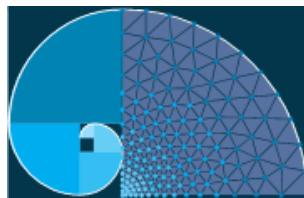
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Phytochemistry, Pharmacological Properties, and Ethnopharmacological Uses of *Glaucium* Mill. (Papaveraceae)

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Review Article

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Abstract

The genus *Glaucium* Mill. (Papaveraceae), commonly referred to as horned poppies, comprises annual and perennial herbaceous plants native to the Mediterranean basin and widely distributed across southern Europe, North Africa, and Western to Central Asia. Several species of the genus have long been incorporated into traditional medical systems for the management of respiratory complaints, pain, inflammatory conditions, skin disorders, and gastrointestinal ailments. This extensive ethnopharmacological background has stimulated increasing scientific interest in the phytochemical composition and pharmacological potential of *Glaucium* species. Phytochemical investigations have demonstrated that *Glaucium* taxa possess a rich and structurally diverse secondary metabolite profile dominated by isoquinoline alkaloids. Aporphine-, protopine-, protoberberine-, and benzophenanthridine-type alkaloids, including glaucine, protopine, allocryptopine, sanguinarine, and chelerythrine, have been extensively identified and are regarded as the principal bioactive constituents of the genus. In addition to alkaloids, *Glaucium* species contain biologically relevant phenolic compounds, particularly flavonoids and phenolic acids, which contribute substantially to antioxidant-related effects. Pharmacological studies, conducted predominantly in vitro and in animal models, indicate that extracts, fractions, and isolated compounds derived from *Glaucium* species exhibit a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, neuroprotective, anticholinesterase, and anticancer-related effects. These activities are largely associated with alkaloid- and polyphenol-rich extracts and often involve multitarget mechanisms. This review critically summarizes current knowledge on the phytochemistry, pharmacological properties, and ethnopharmacological uses of *Glaucium* species. By integrating traditional knowledge with experimental evidence, the review highlights both the pharmacological relevance of the genus and the existing gaps in research, emphasizing the need for further systematic phytochemical, toxicological, and mechanistic studies to better elucidate the therapeutic potential and safety profiles of *Glaucium* taxa.

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1. Introduction

The Papaveraceae family is native primarily to temperate and subtropical regions of the Northern Hemisphere and has many horticultural and pharmaceutical important taxa (Kusman Saygi et al., 2023). Comprising approximately 48 genera and 920 species, Papaveraceae is characterized by high generic endemism, suggesting multiple diversification events within geographically restricted areas. The family exhibits remarkable ecological plasticity, spanning diverse habitats from coastal regions to high-altitude summits, and from arid deserts to forest understories and rocky outcrops (Peng et al., 2023).

Distinguished by high species richness, *Glaucium* Mill. (~28 species) is widely distributed across a broad geography extending from Southern Europe to Southwest Asia (Aykurt et al., 2017). Iran and Türkiye represent major diversity hotspots for the genus (Aslan, 2012). From a biomedical perspective, *Glaucium* species have attracted sustained interest because their traditional use has prompted systematic phytochemical and pharmacological investigations. Studies consistently indicate that isoquinoline alkaloids constitute the dominant and most extensively characterized metabolite class in the genus, while phenolic compounds represent an additional biologically relevant group. In contrast, terpenoid- and lipid-related constituents have been reported only sporadically and generally at minor or trace levels (Hamamcioglu et al., 2018; Akaberi et al., 2021). Alongside chemical profiling, a growing body of experimental evidence has reported diverse biological activities for *Glaucium* extracts and isolated constituents, including antioxidant, anti-inflammatory, antimicrobial, neuroprotective/anticholinesterase, and anticancer-related effects (Akaberi et al., 2021; Kocanci et al., 2022).

In this review, we summarize the current evidence on the distribution, ethnopharmacological relevance, phytochemical composition, and pharmacological activities of *Glaucium* species, with particular emphasis on isoquinoline alkaloids and phenolic constituents as key drivers of the reported bioactivities.

2. Papaveraceae Family and the Genus *Glaucium*

The Papaveraceae family, commonly known as the poppy family, is predominantly distributed across the temperate and subtropical regions of the Northern Hemisphere and comprises numerous species of horticultural and pharmaceutical importance (Kusman Saygi et al., 2023). This family includes approximately 48 genera and 920 species, with more than 70% of the genera being endemic to relatively restricted geographical regions (Peng et al., 2023). Members of Papaveraceae exhibit remarkable ecological adaptability, inhabiting a wide range of environments from sea level to elevations exceeding 6000 m, including forest understories, deserts, rocky cliffs, and alpine screes (Peng et al., 2024).

Within this diverse family, the genus *Glaucium* Mill. represents one of the most species-rich and widely distributed taxa. The genus comprises approximately 28 species, primarily native to southern Europe, the Mediterranean region, and central to south-western Asia (Aykurt et al., 2017). Notably, Iran and Türkiye emerge as major centers of species diversity for *Glaucium*, hosting 17 species and 12 taxa, respectively (Aslan, 2012; Yıldız et al., 2015). Species of the genus are represented by annual or perennial flowering plants characterized by their distinctive aromatic odor and showy flowers, typically displaying yellow, red, or orange coloration. Some *Glaucium* species distributed in Türkiye are shown in Table 1.

According to global distribution data, *Glaucium* species are natively distributed across Europe, Africa, Asia, Tropical Asia, and Australia (WFO, 2025) (Figure 1). Beyond their native ranges, several species have been introduced into non-native regions through human-mediated activities. The brown-colored areas in Figure 1 illustrate these introduced distributions, highlighting regions where *Glaucium* species have spread as a result of anthropogenic dispersal.

Table 1. Some *Glaucium* species distributed in Türkiye with their photographs

Plant species	Plant photographs	Reference
<i>Glaucium alakirensis</i> (endemic)		(Aykurt et al., 2017)
<i>Glaucium acutidentatum</i> (endemic)		(Mungan, 2016)
<i>Glaucium cappadocicum</i> (endemic)		(Mungan, 2016)
<i>Glaucium corniculatum</i> var. <i>corniculatum</i>		(Kusman Saygi et al., 2023).
<i>Glaucium flavum</i>		(Mungan, 2016)
<i>Glaucium grandiflorum</i> subsp. <i>refractum</i> var. <i>torquatum</i> (endemic)		(Kusman Saygi et al., 2023).



Figure 1. Global distribution pattern of the genus *Glaucium* (WFO, 2025). Native ranges are shown in green, while brown-colored areas indicate introduced distributions resulting from anthropogenic dispersal.

3. Phytochemical Composition of *Glaucium* Species

Since antiquity, plants have served as a fundamental source of medicinal agents. They synthesize a wide range of secondary metabolites, such as phenolic compounds, alkaloids, saponins, glycosides, resins, terpenes, and lactones, which play crucial roles in plant growth,

development, and reproduction, as well as in defense against pathogenic challenges (Elshafie et al., 2023). These plant-derived secondary metabolites, commonly referred to as phytochemicals, originate from primary metabolic pathways and exhibit diverse biological and pharmacological activities (Özay et al., 2016).

Phytochemicals are widely distributed throughout the plant kingdom and are present not only in commonly consumed fruits and vegetables but also in numerous medicinal and wild plant species that have long been utilized in traditional healthcare systems. Increasing scientific interest in such non-cultivated and medicinal taxa has revealed that many of these plants represent particularly rich reservoirs of bioactive secondary metabolites with significant therapeutic potential (Alper & Özay, 2022). In this context, medicinal genera traditionally used in folk medicine, such as *Glaucium*, have attracted considerable attention as promising sources of pharmacologically active phytochemicals.

Many *Glaucium* species are traditionally employed as antitussive, analgesic, sedative, and narcotic agents, as well as in the treatment of skin, liver, and inflammatory disorders (Kusman Saygi et al., 2023). This long-standing ethnomedicinal use has stimulated extensive phytochemical investigations, leading to the identification of a rich and structurally diverse secondary metabolite profile within the genus. Although alkaloids have been the primary focus of most phytochemical studies on *Glaucium*, several investigations have also confirmed the presence of other phytochemical classes, including ketones, glycosides, fatty acids, alkenes, terpenes, and fatty alcohols, generally detected in minor or trace amounts (Hamamcioğlu et al., 2018). Among these constituents, alkaloids clearly represent the most abundant, chemically diverse, and biologically significant secondary metabolite class in the genus, forming the basis of many of its reported pharmacological effects.

3.1. Alkaloids

Alkaloids are secondary metabolites that exhibit a high degree of diversity not only in terms of their botanical and biochemical origins but also with respect to their chemical structures and pharmacological effects. Accordingly, several classification approaches have been proposed. Alkaloids may be classified based on their molecular precursors, core structural skeletons, biosynthetic origins, or the biochemical pathways involved in their formation (Dey et al., 2020).

In general, alkaloids are naturally occurring plant compounds containing at least one nitrogen atom, typically basic in character and structurally complex. A major milestone in alkaloid research was the isolation of the first alkaloid, morphine, from *Papaver somniferum* in 1804 (Dey et al., 2020). Based on their chemical structures, alkaloids are grouped into isoquinoline, indole, pyridine/piperidine, pyrrolidine, purine, imidazole, steroid, and diterpenoid alkaloids (Yang et al., 2024). Within this framework, the genus *Glaucium* is distinguished by its richness in isoquinoline alkaloids, which represent the dominant and most extensively investigated secondary metabolite class in the genus (Akaberi et al., 2021). In a study on the aerial parts of *G. corniculatum*, six isoquinoline alkaloids—dehydroberberine, berberine, protopine, allocryptopine, corydine, and glaucine—were identified (Al-Saleem et al., 2024).

Alkaloids such as glaucine, protopine, isoboldine, allocryptopine, scoulerine, and corydine are of particular importance in *Glaucium* species, as they are both pharmacologically active and serve as chemotaxonomic markers for interspecific differentiation (Figure 2). These compounds have been extensively analyzed in species including *G. corniculatum*, *G. flavum*, *G. grandiflorum*, and *G. oxylobum*, with their presence and quantitative profiles confirmed using advanced analytical techniques such as HPLC and LC-MS/MS (Bournine et al., 2013a; Chang et al., 2015; Morteza-Semnani et al., 2005).

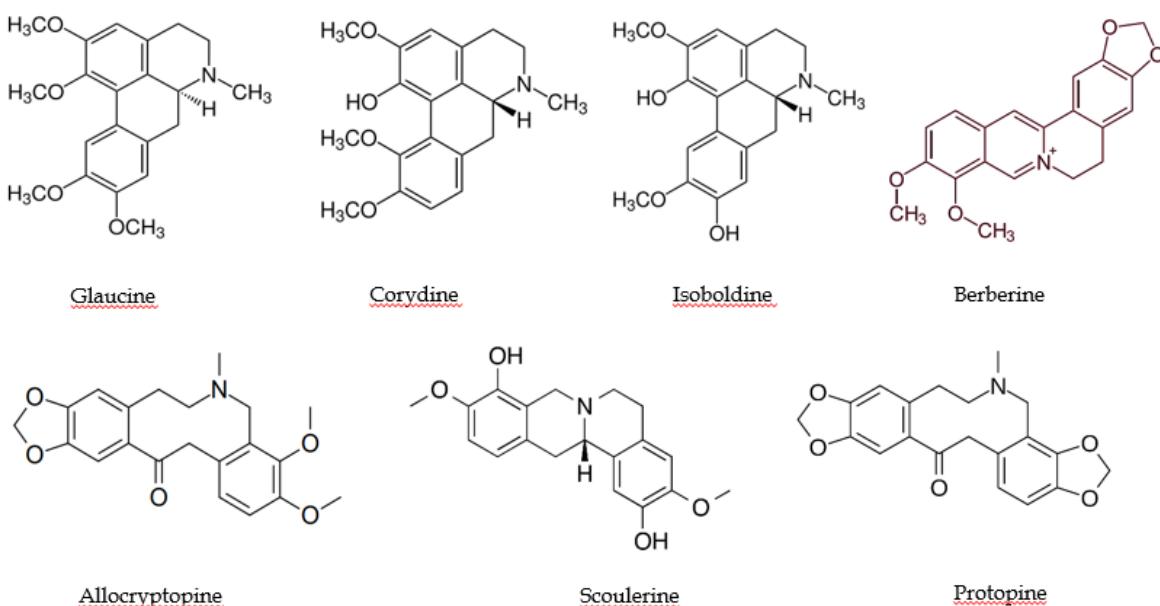


Figure 2. Major isoquinoline alkaloids most frequently reported in *Glaucium* species

Comprehensive reviews and recent analytical studies indicate that aporphine alkaloids predominate in the genus *Glaucium*, while other structural types—such as protopine, benzophenanthridine, benzylisoquinoline, protoberberine, and morphinan alkaloids—are also characteristic of the genus (Akaberi et al., 2021; Yagi et al., 2024). The aporphine alkaloid glaucine, first isolated from *G. flavum*, is widely distributed among *Glaucium* species and is used clinically as an antitussive agent; moreover, it has been reported to exhibit notable anticancer activity (Yagi et al., 2024).

GC-MS and chromatographic studies conducted on different *Glaucium* species and populations (e.g., *G. flavum*, *G. leiocarpum*, *G. corniculatum*, and *G. grandiflorum*) have revealed the presence of numerous alkaloids, including glaucine, isocorydine, protopine, allocryptopine, and dehydroberberine, as well as novel structures such as glauciumoline (Bozkurt et al., 2022; Demir et al., 2017; Kurkin et al., 2024; Kusman Saygi et al., 2023). The pronounced chemotypic and geographic variations observed in alkaloid profiles highlight the decisive roles of environmental conditions and genetic factors in shaping the phytochemical composition of the genus *Glaucium* (Bozkurt et al., 2024; Petitto et al., 2010).

3.2. Phenolic compounds

In addition to alkaloids, phenolic compounds constitute another major and biologically relevant class of secondary metabolites in *Glaucium* species, as demonstrated by several phytochemical investigations employing chromatographic and mass spectrometric techniques (Al-Saleem et al., 2024). Plant phenolics comprise a structurally diverse group of compounds characterized by one or more phenolic hydroxyl groups and are known to play essential roles in plant defense mechanisms, pigmentation, growth regulation, and responses to biotic and abiotic stress factors (Ozay et al., 2016). From a chemical perspective, phenolic compounds may occur as glycosides or aglycones, exist in free or matrix-bound forms, and appear as monomeric or polymeric structures, reflecting their substantial structural and functional diversity (Alara et al., 2021). Based on their core structures, phenolic compounds are commonly classified into phenolic acids, coumarins, flavonoids, stilbenes, lignans, and tannins (Ozay et al., 2024).

Phytochemical studies have shown that *Glaucium* species contain a broad spectrum of phenolic constituents, predominantly represented by flavonoids and phenolic acid derivatives (Figure

3), particularly hydroxycinnamic acid-related compounds (Kusman Saygi et al., 2023). These phenolics are mainly detected in the aerial parts of the plants, such as leaves, flowers, and stems, and are frequently present in glycosylated forms, a feature known to influence their solubility, stability, and biological activity (Hamamcioglu et al., 2018). Owing to these properties, phenolic compounds substantially contribute to the antioxidant capacity of *Glaucium* extracts by enhancing free radical scavenging potential.

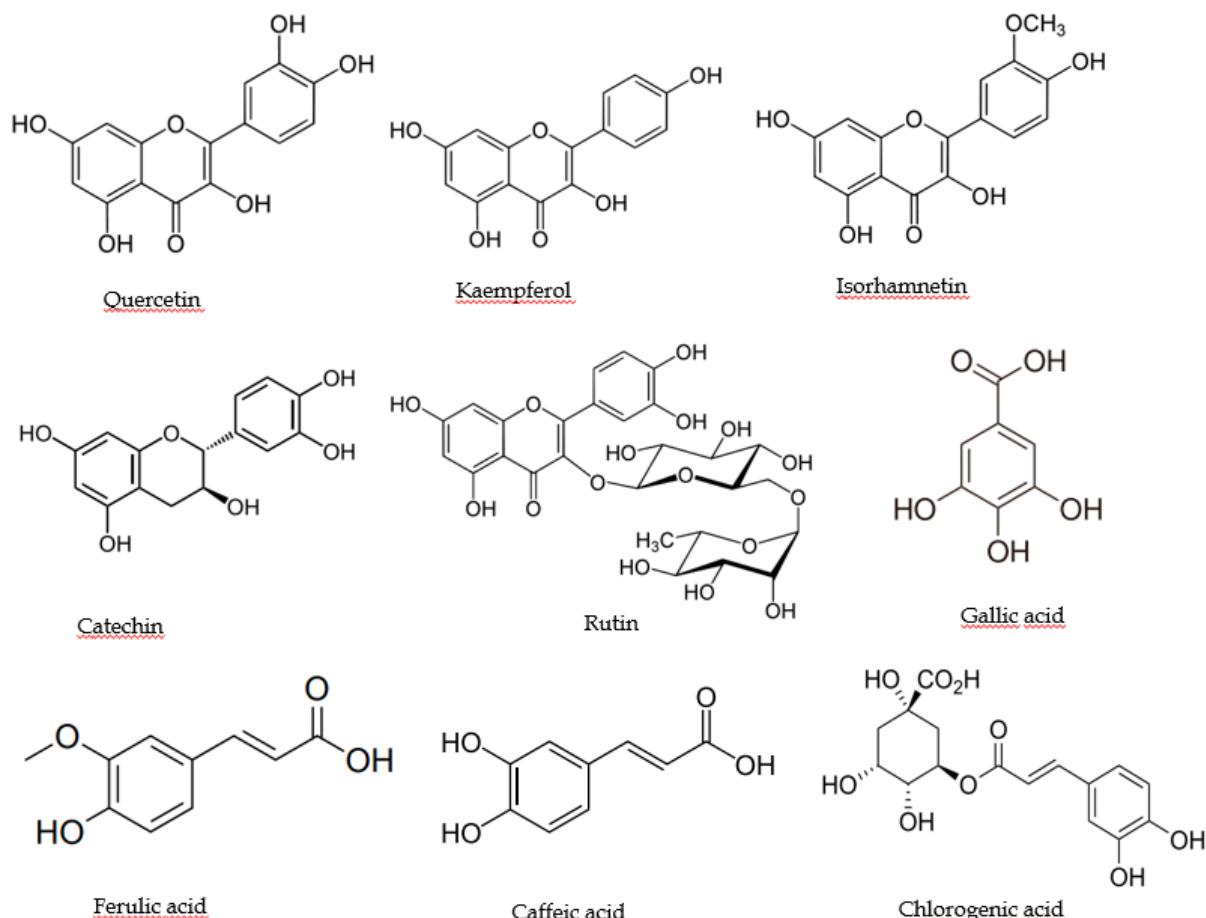


Figure 3. Major flavonoids and phenolic acids most frequently identified in *Glaucium* species

Species-specific investigations further illustrate the diversity of phenolic profiles within the genus. In *G. flavum*, seven phenolic compounds—kaempferol, caffeic acid, catechin hydrate, syringic acid, chlorogenic acid, isoquercitrin, and trans-hydroxycinnamic acid—have been identified (Boulaaba et al., 2019). Similarly, analyses of *G. alakirensis* (endemic) revealed the presence of gallic acid, catechin, chlorogenic acid, hydroxybenzoic acid, and quercetin, indicating a phenolic composition dominated by flavonoids and phenolic acids (Özcandır et al., 2024). In *G. acutidentatum*, coumaric acid and ferulic acid derivatives have been reported, alongside a flavonoid profile largely occurring in glycosylated form; only quercetin and isorhamnetin were detected as aglycones in methanolic extracts, whereas glycosides of quercetin, isorhamnetin, and kaempferol were more prevalent (Yagi et al., 2024). A detailed study on the aerial parts of *G. corniculatum* identified six flavonoids—quercetin, isorhamnetin, rutin, isorhamnetin-3-O-rutinoside, quercetin-3-O-glucoside-7-O-rutinoside, and isorhamnetin-3-O-glucoside-7-O-rutinoside—together with two hydroxycinnamic acid malate esters, namely phasic acid and 2-O-feruloyl-L-malate, further highlighting the phenolic diversity of the genus (Al-Saleem et al., 2024).

Coumarins have also been reported within the phenolic fraction of *Glaucium*, although they appear to be minor and sporadically distributed constituents rather than dominant metabolites. Recent metabolomic investigations on *G. acutidentatum* detected coumarins alongside flavonoids and organic acids; however, these compounds were present within extract chemotypes clearly dominated by isoquinoline alkaloids such as glaucine (Yagi et al., 2024). In line with these findings, Akaberi et al. (2021) regard coumarins in *Glaucium* as underexplored minor components, for which no consistent structural series has been established across the genus and whose pharmacological profiles remain comparatively limited when contrasted with the extensively documented antioxidant, enzyme-inhibitory, and cytotoxic activities of alkaloids.

Overall, beyond alkaloids, *Glaucium* species exhibit a chemically rich phenolic profile dominated by flavonoids and phenolic acids, with contributions from coumarins and related compounds. This phenolic diversity, revealed primarily through modern LC-MS-based profiling approaches, underpins many of the antioxidant and complementary bioactivities reported for the genus and highlights phenolic compounds as important, albeit secondary, contributors to the pharmacological potential of *Glaucium* species.

4. Pharmacological Activities of *Glaucium* Species

Pharmacological studies conducted on *Glaucium* species have demonstrated that this genus exhibits a broad spectrum of biological activities, largely attributable to its phytochemical profile enriched in isoquinoline alkaloids (Akaberi et al., 2021). The available literature indicates that extracts, fractions, and isolated compounds obtained from various *Glaucium* species—particularly *G. flavum*, *G. grandiflorum*, *G. acutidentatum*, and *G. corniculatum*—possess notable pharmacological potential, especially with respect to anticholinesterase, antioxidant, and neuroprotective activities (Bournine et al., 2013b; Hamamcioğlu et al., 2018). In addition, a growing body of evidence has reported further biological effects of these species, including anticancer, antiproliferative, antimicrobial, and anti-inflammatory activities, underscoring the pharmacological relevance of the genus (Alsheikh et al., 2024; Altın, 2025).

4.1. Neuroprotective and anticholinesterase effects

Cell-based studies have consistently demonstrated that *Glaucium* extracts exert direct neuroprotective effects, particularly under conditions of oxidative stress. Methanolic and aqueous extracts of *G. corniculatum*, rich in flavonoids such as rutin and quercetin, were shown to prevent hydrogen peroxide (H_2O_2)-induced loss of viability in nerve growth factor (NGF)-differentiated PC12 cells while significantly preserving neurite number and length. In parallel, these extracts suppressed oxidative stress-induced acetylcholinesterase (AChE) upregulation at the mRNA level, whereas methanolic extracts additionally reduced AChE protein expression, highlighting a close association between flavonoid content and the observed neuroprotective activity (Kocancı et al., 2022). Beyond flavonoid-mediated effects, alkaloid-rich extracts have provided deeper mechanistic insights into *Glaucium*-derived neuroprotection. Allocryptopine-rich alkaloid extracts from *G. corniculatum* effectively suppressed oxidative stress-induced apoptosis in differentiated PC12 cells by reducing intracellular reactive oxygen species (ROS) levels, downregulating pro-apoptotic markers (Bax and caspase-3/9), upregulating the anti-apoptotic protein Bcl-2, and normalizing cell cycle distribution. These findings support a mitochondria-associated mechanism underlying the neuroprotective effects of *Glaucium* alkaloids (Dolanbay et al., 2021).

Comparable neuroprotective responses have been reported for *G. acutidentatum* and *G. corniculatum* using NGF-differentiated PC12 (dPC12) cells exposed to H_2O_2 -induced oxidative stress. In these models, both alkaloid-rich methanolic and aqueous extracts exhibited pronounced anticholinesterase activity, producing 35–90% inhibition of AChE in vitro and significantly reducing cellular AChE activity. Concomitantly, the extracts attenuated oxidative

cytotoxicity in a dose-dependent manner. Notably, aqueous extracts of *G. corniculatum* exerted stronger neuroprotective effects than methanolic extracts, with maximal protection observed at concentrations of 500–1000 μ g/mL, whereas extracts of *G. acutidentatum* displayed significant but comparatively moderate protection up to 500 μ g/mL. All tested concentrations conferred statistically significant protection relative to the oxidative stress control, with maximal effects observed at higher extract doses (Kocancı et al., 2017a). Further support for the neuroprotective profile of *G. acutidentatum* was obtained from studies demonstrating that methanolic and aqueous extracts from its aerial parts significantly attenuated oxidative stress-induced cytotoxicity in dPC12 cells within the 100–500 μ g/mL concentration range. In addition to preserving cell viability, these extracts promoted neurite outgrowth and modulated inflammatory responses by suppressing the pro-inflammatory cytokine IL-6 while enhancing levels of the anti-inflammatory cytokine IL-10. Collectively, these findings indicate that *G. acutidentatum* extracts exert neuroprotective effects through combined antioxidant and anti-inflammatory mechanisms (Hamamcioğlu et al., 2018).

A consistent pharmacological hallmark of *Glaucium* species is potent cholinesterase inhibition, which complements their direct neuroprotective effects. In this context, purified isoquinoline alkaloids—including protopine, allocryptopine, and glaucine—isolated from the aerial parts of the endemic species *G. cappadocicum* were evaluated for anticholinesterase activity in vitro. Among these compounds, protopine exhibited the strongest AChE inhibitory activity (78.32% inhibition, $IC_{50} = 31.4 \mu$ g/mL), whereas allocryptopine and glaucine showed moderate inhibition, underscoring the contribution of specific alkaloid scaffolds to enzyme-targeted neuroprotection (Altin, 2025). Similarly, extracts obtained from the aerial parts of *G. alakirensis* demonstrated marked anticholinesterase activity, with the methanolic extract exhibiting the highest AChE inhibition (70.1%, $IC_{50} = 41.3 \mu$ g/mL) compared with ethyl acetate and n-hexane extracts. These results further suggest that polar extraction favors enrichment of bioactive cholinesterase-inhibiting constituents in *Glaucium* species (Delik et al., 2024).

At the level of isolated metabolites, isoquinoline alkaloids from *G. arabicum*, including glaucine, protopine, isocorydine, and norsanguinarine, were identified as potent cholinesterase inhibitors. Using the Ellman assay, glaucine showed the strongest AChE inhibition (84.1%, $IC_{50} = 9.6 \mu$ M), while protopine exhibited the highest BChE inhibitory activity (71.4%, $IC_{50} = 13.3 \mu$ M), supporting the dual cholinesterase inhibitory potential of *Glaucium*-derived alkaloids (Elbermawi et al., 2018). Consistent findings were reported for alkaloid fractions obtained from *G. corniculatum* var. *corniculatum* and *G. grandiflorum* subsp. *refractum* var. *torquatum*, which yielded several isoquinoline alkaloids, including sanguinarine and chelerythrine. Among these, sanguinarine exhibited the most potent AChE inhibition (87.6%, $IC_{50} = 8.4 \mu$ M), whereas glaucine showed notable BChE inhibitory activity. These results further highlight benzophenanthridine- and aporphine-type alkaloids as key contributors to the neuropharmacological profile of *Glaucium* species (Kusman Saygı et al., 2023).

Finally, multitarget neuroprotective activity has been demonstrated for *G. grandiflorum* var. *grandiflorum* and *G. corniculatum* subsp. *refractum*. A methanolic extract of *G. grandiflorum* exhibited strong AChE inhibition (~81% at 320 μ g/mL), comparable to galantamine, and provided protection against oxidative DNA damage (Ozsoy et al., 2018). In parallel, an alkaloid-rich extract of *G. corniculatum*, characterized by high allocryptopine and protopine content, showed potent inhibitory activity against both AChE and BChE and additionally inhibited prolyl oligopeptidase, an enzyme implicated in neurodegenerative processes. These findings underscore the multitarget neuropharmacological potential of *Glaucium*-derived alkaloids (Bozkurt et al., 2024).

4.2. Antioxidant activity

Members of the genus *Glauicum* exhibit pronounced antioxidant potential, which is primarily attributed to their rich phenolic composition and, in certain taxa, may be further modulated by alkaloid-containing fractions. Accumulating evidence from in vitro studies indicates that extracts obtained from different *Glauicum* species effectively scavenge free radicals, enhance reducing capacity, and modulate redox homeostasis through multiple complementary mechanisms. In *G. flavum*, phenolic-enriched fractions containing compounds such as kaempferol, caffeic acid, catechin hydrate, syringic acid, and chlorogenic acid demonstrated strong antioxidant performance across several assays, including total antioxidant capacity, ferric reducing antioxidant power (FRAP), β -carotene bleaching inhibition, and free radical scavenging activity (Boulaaba et al., 2019). Beyond conventional extracts, green-synthesized gold nanoparticles (AuNPs) produced using *G. flavum* leaf extract were also reported to exhibit significant antioxidant activity alongside low cytotoxicity and acceptable biocompatibility, underscoring the contribution of *Glauicum*-derived phytochemicals to innovative antioxidant applications (Dehghani et al., 2023).

Consistent findings have been reported for *G. grandiflorum var. grandiflorum*, whose methanolic extract displayed robust antioxidant activity in multiple in vitro models. These effects were evidenced by inhibition of lipid peroxidation, strong 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity, high FRAP reducing power, and pronounced hypochlorous acid scavenging, collectively reflecting broad redox-modulatory properties (Ozsoy et al., 2018).

The influence of extract polarity on antioxidant performance was further illustrated in *G. cappadocicum*. Extracts obtained from the aerial parts using solvents of different polarities were evaluated using DPPH radical scavenging and FRAP reducing power assays, alongside spectrophotometric determination of total phenolic and flavonoid contents. Methanolic and ethyl acetate extracts exhibited strong free radical scavenging and reducing capacities, which closely paralleled their high phenolic and flavonoid contents, whereas n-hexane extracts showed limited antioxidant activity. Overall, these results suggest that *G. cappadocicum* may represent a promising natural antioxidant source in oxidative stress-related biological processes (Altin, 2025).

A more comprehensive assessment was conducted for *G. acutidentatum*, in which the impact of different extraction techniques—maceration, homogenizer-assisted extraction, and infusion—on antioxidant activity was systematically examined. Antioxidant capacity was evaluated using a battery of in vitro assays, including DPPH and ABTS radical scavenging, FRAP and CUPRAC reducing power, and metal chelating activity. Methanolic extracts obtained via homogenizer-assisted extraction exhibited the highest total phenolic content and the strongest overall antioxidant performance, with elevated Trolox equivalent values and superior Fe^{3+} and Cu^{2+} reducing capacities. LC-MS/MS analyses revealed the presence of flavonoids, phenolic acids, and several isoquinoline alkaloids, particularly glaucine, and the authors emphasized that the observed antioxidant activity may arise from the combined contribution of phenolic constituents and alkaloids (Yagi et al., 2024).

Similarly, the endemic species *G. alakirensis* demonstrated notable antioxidant potential when evaluated using ethanolic extracts. HPLC analysis of the phenolic profile identified gallic acid, chlorogenic acid, catechin, quercetin, and hydroxybenzoic acid as major constituents. Functional antioxidant assays revealed a high, concentration-dependent DPPH radical scavenging capacity, which notably exceeded that of caffeic acid and rosmarinic acid used as positive controls. Furthermore, measurements of total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) indicated high TAS and low OSI values, reinforcing the view that *G. alakirensis* represents a strong natural antioxidant source with potential relevance in mitigating oxidative stress (Özcandır et al., 2024).

4.3. Anti-inflammatory activity

Anti-inflammatory properties of *Glaucium* species have been demonstrated across multiple experimental levels, including enzyme-based assays, cell culture models, and in vivo systems. At the enzyme level, the methanolic extract of *G. grandiflorum* var. *grandiflorum* inhibited cyclooxygenase-2 (COX-2) activity by approximately 69%, highlighting its relevance to inflammatory pathways and cancer chemoprevention (Ozsoy et al., 2018). Complementary mechanistic evidence was provided by studies on alkaloid extracts of *G. corniculatum* subsp. *refractum*, containing allocryptopine, tetrahydropalmatine, and tetrahydroberberine N-oxide. In BV-2 microglial cells, these alkaloid-rich extracts markedly suppressed lipopolysaccharide (LPS)-induced ROS production and downregulated the expression of iNOS, COX-2, and pro-inflammatory cytokines (TNF- α , IL-1 β , and IL-6) through inhibition of the p38 MAPK signaling pathway, indicating a substantial attenuation of neuroinflammatory responses (Dolanbay et al., 2021). Collectively, these findings complement the strong in vitro antioxidant profiles reported for *Glaucium* species and suggest a dual antioxidant-anti-inflammatory mode of action that may be relevant to neurodegenerative and inflammatory disorders.

Further evidence for cell-based neuroprotective and anti-inflammatory activity has been obtained from studies on methanolic and aqueous extracts of *G. corniculatum* using a hydrogen peroxide (H₂O₂)-induced oxidative stress model in NGF-differentiated PC12 (dPC12) cells. Phytochemical analyses revealed rutin and quercetin as the major flavonoid constituents of both extracts, with higher concentrations detected in the methanolic extract. Functional assays demonstrated that the extracts were non-cytotoxic within the concentration range of 100–500 μ g/mL and significantly attenuated oxidative stress-induced loss of cell viability. In parallel, both extracts suppressed the release of the pro-inflammatory cytokine interleukin-6 (IL-6) and dose-dependently increased levels of the anti-inflammatory cytokine interleukin-10 (IL-10), with the most pronounced effects observed for the aqueous extract at 500 μ g/mL. Notably, this extract reduced IL-6 secretion by 79-fold relative to the H₂O₂-treated group and increased IL-10 secretion by 87-fold compared with untreated controls. These results indicate that rutin- and quercetin-rich extracts of *G. corniculatum* mitigate oxidative stress-associated neuronal damage through anti-inflammatory mechanisms and exhibit marked neuroprotective potential (Koçancı et al., 2017b).

Consistent with these in vitro observations, the anti-inflammatory activity of *G. grandiflorum* has also been confirmed in vivo using the carrageenan-induced hind paw edema model in Wistar albino rats. Intraperitoneal administration of the methanolic extract of aerial parts (leaves, stems, and flowers) at doses ranging from 20 to 200 mg/kg resulted in a significant, dose-dependent inhibition of paw edema. Notably, at 200 mg/kg, the extract exerted stronger inhibitory effects during the late phase of inflammation than the reference drug indomethacin (8–10 mg/kg), with statistically significant differences. These findings suggest that *G. grandiflorum* displays pronounced anti-inflammatory activity, particularly during the prostaglandin-mediated phase of inflammation, and underscore its potential as a source of bioactive anti-inflammatory compounds (Morteza-Semnani et al., 2002).

Finally, anti-inflammatory activity has also been demonstrated for *G. flavum* in macrophage-based models. Extracts obtained from the aerial parts of the plant, particularly the ethyl acetate (EA) fraction, were evaluated in LPS-stimulated RAW 264.7 murine macrophages. Treatment with the EA fraction at concentrations of 12.5–100 μ g/mL significantly suppressed nitric oxide (NO) production in a concentration-dependent manner, with the strongest inhibition observed at 50 and 100 μ g/mL. In contrast, petroleum ether and ethanol fractions exhibited comparatively weaker effects. These results indicate that phenolic-enriched fractions of *G. flavum*, especially the EA fraction, exert potent anti-inflammatory activity, likely through the inhibition of LPS-induced NO production in activated macrophages (Boulaaba et al., 2019).

4.4. Cytotoxic and anticancer activities

A growing body of evidence indicates that *Glaucium* species are a rich source of bioactive alkaloids, particularly isoquinoline derivatives, which exhibit antiproliferative and pro-apoptotic effects in various cancer cell models. Alkaloid-rich extracts of *G. acutidentatum* and *G. corniculatum* have been shown to exert dose-dependent antiproliferative activity against HT-29 colon and HeLa cervical cancer cell lines while concurrently retaining acetylcholinesterase inhibitory activity, suggesting their potential relevance for multitarget therapeutic strategies (Kocancı et al., 2017a). In addition, aqueous and methanolic extracts of *G. acutidentatum* demonstrated selective cytotoxicity toward human hypopharyngeal carcinoma cells, with minimal effects on other tested cell lines, further supporting a degree of tumor selectivity (Yagi et al., 2024).

Among the investigated species, *G. flavum* has emerged as one of the most extensively studied taxa with notable anticancer-related properties. Phenolic-rich fractions obtained from the shoots of *G. flavum* significantly inhibited the proliferation of MCF-7 breast cancer cells, with ethyl acetate fractions exhibiting the strongest activity alongside pronounced antioxidant and anti-inflammatory effects (Boulaaba et al., 2019). Furthermore, alkaloid extracts derived from the roots of *G. flavum* inhibited breast cancer cell growth in vitro and reduced both tumor growth and vascularization in a human glioma chorioallantoic membrane model, with protopine identified as a major constituent (Bournine et al., 2013b). Subsequent quantitative analyses revealed the presence of protopine and bocconoline, the latter displaying potent and relatively selective cytotoxicity against breast cancer cells ($IC_{50} = 7.8 \mu M$) with low toxicity toward normal cells, implicating bocconoline as a key contributor to the observed anticancer effects (Bournine et al., 2013a). Consistently, studies conducted on A549 lung cancer cells reported reduced cell viability following *G. flavum* extract treatment, accompanied by upregulation of pro-apoptotic genes (p53, Bax, and Bad) and downregulation of the anti-apoptotic marker Bcl-2, indicating apoptosis induction via intrinsic pathways (Kalantari & Entezari, 2020).

The antiproliferative potential of endemic *G. cappadocicum* has also been explored. Extracts prepared from the aerial parts of this species exhibited measurable antiproliferative activity, prompting the isolation and characterization of the secondary metabolites responsible for these effects. As a result, several isoquinoline alkaloids—namely salutaridine, glaucine, norglaucine, isocorydine, norisocorydine, isoboldine, predicentrine, and corytuberine—were isolated from this species for the first time. The isolated alkaloids were evaluated against A549 lung and HT-29 colon cancer cell lines within a concentration range of 5–100 μM , with corytuberine exhibiting the most pronounced cytotoxic effect against A549 cells ($IC_{50} = 61.2 \mu M$) (Altin, 2025).

In addition to classical antiproliferative studies, phytochemical investigation of the aerial parts of *G. arabicum* Fresen. led to the isolation of two previously undescribed isoquinoline alkaloids, araglaucine A and araglaucine B, along with several known alkaloids, including araglaucine C, *N*-methylcanadinium-, *N*-methylstylopine-, and protopine-type derivatives, as well as norsanguinarine. The biological activities of these compounds were evaluated using B16 melanoma cell lines. Among the tested molecules, (7R,14S)-trans-*N*-methylcanadinium nitrate showed notable inhibition of melanin synthesis (approximately 35%) at 5 $\mu g/mL$ (12.01 μM), while exhibiting relatively low cytotoxicity (approximately 12%). Although the primary endpoint focused on anti-melanogenic activity rather than direct growth inhibition, these findings suggest that *Glaucium*-derived isoquinoline alkaloids may act as modulators of melanoma-associated cellular processes rather than classical cytotoxic anticancer agents (Elbermawi et al., 2018).

Finally, isoquinoline alkaloids isolated from the aerial parts of *G. corniculatum* var. *corniculatum* and *G. grandiflorum* subsp. *refractum* var. *torquatum* have been systematically evaluated for their cytotoxic potential in vitro. Following methanolic extraction and purification, major

alkaloids—including chelerythrine, sanguinarine, glaucine, and protopine—were tested against HeLa (cervical cancer) and MCF-7 (breast cancer) cell lines using the MTT assay. Chelerythrine isolated from *G. corniculatum* exhibited the most pronounced cytotoxic activity, with an IC_{50} value of 11.2 μ M against HeLa cells, whereas sanguinarine also demonstrated notable antiproliferative effects. In contrast, glaucine and protopine showed weaker but concentration-dependent cytotoxicity. Importantly, these results indicated that benzophenanthridine-type isoquinoline alkaloids possess stronger cytotoxic potential than aporphine-type derivatives, highlighting chelerythrine and sanguinarine as promising bioactive scaffolds for further investigation in anticancer drug discovery research (Kuşman Saygı et al., 2023).

4.5. Antimicrobial activity

Several studies have investigated the antibacterial and antifungal properties of different *Glaucium* species, revealing marked interspecific variability that appears to be strongly influenced by extraction methods, solvent polarity, and phytochemical composition.

The antibacterial and antifungal potential of the total methanolic extract and the alkaloid sub-fraction obtained from the flowering aerial parts of the Iranian endemic species *G. vitellinum* was evaluated in vitro by Mehrara et al. (2015). Antibacterial activity was assessed using the agar well diffusion method against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, while minimum inhibitory concentration (MIC) values were determined by the microdilution assay. Both the crude methanolic extract and, more prominently, the alkaloid sub-fraction exhibited marked antibacterial activity against *S. aureus* and *S. typhi*, with the alkaloid fraction showing stronger efficacy (MIC values of 0.9 mg/mL and 11.7 mg/mL, respectively). In contrast, only limited activity was observed against Gram-negative bacteria, including *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Antifungal evaluation using the disk diffusion method demonstrated no significant inhibition against tested dermatophytes (e.g., *Aspergillus flavus* and *Trichophyton mentagrophytes*); however, *Candida albicans* exhibited high sensitivity, particularly to the alkaloid sub-fraction, with an MIC value (0.02 mg/mL) comparable to that of nystatin. These findings suggest that the antifungal activity of *G. vitellinum* is largely attributable to its isoquinoline alkaloid content (Mehrara et al., 2015).

Similar antimicrobial investigations have been conducted on *G. alakirensis*, where antibacterial and antifungal activities of ethanol extracts were tested against *S. aureus*, *Enterococcus faecalis*, *E. coli*, *P. aeruginosa*, *Candida albicans*, and *Candida tropicalis*. The extracts were active against *E. coli*, *C. albicans*, and *C. tropicalis* at 50 μ g/mL, while higher concentrations were required to inhibit *S. aureus* and *E. faecalis* (100 μ g/mL) and *P. aeruginosa* (200 μ g/mL), indicating a concentration-dependent antimicrobial response (Özcandır et al., 2024).

Broader comparative studies further support the influence of solvent polarity on antimicrobial efficacy. Methanolic and chloroform extracts from the aerial parts of *G. grandiflorum* Boiss. & A. Huet, *G. oxylobum* Boiss. & Buhse, and *G. paucilobum* demonstrated concentration-dependent antibacterial activity against *S. aureus*, *Streptococcus sanguinis*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. While methanolic extracts exhibited selective activity, particularly against Gram-negative bacteria, chloroform extracts showed broader and stronger antibacterial effects across all tested strains, likely due to their enhanced capacity to solubilize non-polar bioactive constituents (Morteza-Semnani et al., 2005). In addition, the methanolic extract of *G. grandiflorum* var. *grandiflorum* was reported to be effective against *Candida krusei* (Tosun et al., 2006), and methanolic extracts of *G. elegans* displayed antibacterial activity against *S. aureus*, *E. coli*, *Salmonella enteritidis*, *Bacillus anthracis*, and *Proteus* species (Soureshjan & Heidari, 2014).

Conversely, not all studies report strong antimicrobial effects within the genus. In an evaluation of *G. vitellinum*, Hakemi Vala et al. (2017) observed no detectable inhibition zones

for methanolic extracts or alkaloid fractions in disk diffusion assays. MIC analyses further indicated that methanolic extracts were ineffective against Gram-negative bacteria, whereas the alkaloid fraction exhibited selective activity solely against *Salmonella typhimurium*. Collectively, these findings highlight the pronounced variability in antimicrobial efficacy among *Glaucium* species and underscore the critical role of phytochemical diversity and extract type in determining the antimicrobial spectrum.

4.6. Antidiabetic-related effects and enzyme inhibition

Recent studies have explored the potential of various *Glaucium* species to modulate diabetes-associated biochemical processes, particularly through the attenuation of oxidative stress and the inhibition of carbohydrate-digesting enzymes. The hydroalcoholic extract prepared from the aerial parts of *G. flavum* was evaluated in alloxan-induced diabetic male Wistar rats. Hyperglycemia was induced by a single dose of alloxan (120 mg/kg), followed by oral administration of the extract at 500 mg/kg (body weight basis). Treatment with the *G. flavum* extract significantly increased the activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), in both heart and brain tissues compared with the diabetic control group. Notably, the enhancement of antioxidant enzyme activities was more pronounced in brain tissue and, for certain parameters, exceeded the effects observed in the glibenclamide-treated group (5 µg/kg). These findings suggest that *G. flavum* extract primarily mitigates hyperglycemia-associated oxidative stress and strengthens endogenous antioxidant defenses, rather than acting as a direct hypoglycemic agent (Khoshvaghti et al., 2019).

Complementary evidence has been provided by a recent in vitro study examining the antidiabetic-related enzyme inhibitory potential of different extracts obtained from the aerial parts of *G. acutidentatum*. In this study, α -amylase and α -glucosidase inhibition assays revealed that methanolic extracts obtained by homogenizer-assisted extraction (HAE-M) and maceration (MAC-M) exhibited significantly stronger inhibitory activity than aqueous extracts. Among these, the HAE-M extract demonstrated the highest α -amylase inhibitory activity, while methanolic extracts overall showed superior enzyme suppression capacity. The enhanced inhibitory effects were attributed to the richer phytochemical composition of methanolic extracts, particularly their flavonoid and phenolic content, which are known to interfere with carbohydrate-digesting enzymes. Although these findings are limited to in vitro conditions, the observed α -amylase and α -glucosidase inhibition indicates that *G. acutidentatum* may contribute to the modulation of postprandial glucose metabolism, highlighting its potential as a natural source of enzyme inhibitors relevant to diabetes management (Yagi et al., 2024).

5. Ethnopharmacological Uses of *Glaucium* Species

Across the Mediterranean and West Asian regions, *Glaucium* (Papaveraceae) species have been widely used in folk medicine for respiratory complaints, particularly cough, as well as for sedative-hypnotic, narcotic, analgesic, gastrointestinal (laxative), and dermatological purposes. In certain regions, additional uses including diuretic, renal, antihemorrhoidal, and memory-related applications have also been reported (Mehdiyeva et al., 2025). Among the genus, *G. corniculatum* and *G. flavum* are the most frequently cited species in ethnobotanical surveys conducted across different geographical areas (Mehdiyeva et al., 2025; Zadali et al., 2022).

In Türkiye, ethnobotanical and pharmacognostic studies indicate that *Glaucium* species have traditionally been employed in the treatment of headaches, eye disorders, wound healing, joint pain, constipation, and liver-related ailments. These uses are consistent with their reported antitussive, sedative, narcotic, analgesic, and antihemorrhoidal properties, as well as their

application in various skin disorders (Bozkurt et al., 2022; Yagi et al., 2024). The ethnobotanical uses of *Glaucium* species in Türkiye are summarized in Table 2. Experimental studies further suggest that *Glaucium* taxa exhibit antimicrobial, anti-inflammatory, antitumoral, and analgesic activities, which are largely attributed to their rich secondary metabolite profiles, including isoquinoline alkaloids (Hamamcioğlu et al., 2018).

Species-specific ethnobotanical records highlight distinct regional practices. *G. grandiflorum*, native to the Eastern Mediterranean and Iran, has been traditionally used in Türkiye for blood purification and the treatment of eye diseases (Özsoy et al., 2018). *G. corniculatum* subsp. *corniculatum* and *G. leiocarpum* are widely known in Türkiye for their sedative and antitussive effects; in some regions, their leaves are also consumed as food, particularly as pastry fillings (Saday, 2009; Deniz et al., 2010). Outside Türkiye, *G. flavum* has a long history of use in Sicily, especially in the Aegadian Islands, where internal administration was limited due to potential nervous system effects, while external application was preferred. In this context, the species has been traditionally used for skin disorders and, more distinctively, for the topical treatment of hematomas, representing a culturally specific therapeutic practice (La Rosa et al., 2021).

Given the high alkaloid content of *Glaucium* species, their ethnomedicinal applications are predominantly associated with central nervous system, respiratory, and pain-related indications. Nevertheless, these traditional uses should be interpreted strictly within the framework of folk medicine rather than clinical efficacy, considering the potential toxicity and variability related to dosage and preparation methods.

Table 2. Ethnomedicinal Uses of *Glaucium* Species in Türkiye

Plant species	Turkish common name	Plant part used	Traditional use (indication)	Preparation/administration	Reference
<i>Glaucium leiocarpum</i>	Boynuzlu gelincik	Leaves, stem	Antitussive; sedative (sleep-promoting) Headache	Decoction (herbal tea) Smoked in cigarette form (Erbay et al., 2018)	(Deniz et al., 2010)
<i>Glaucium leiocarpum</i>	Boynuzlu gelincik	Leaves	Wound healing	Crushed with olive oil	(Bulut et al., 2017)
<i>Glaucium leiocarpum</i>	Boynuzlu gelincik	Leaves	Food use	Cooked by frying with onion	(Eşen, 2008)
<i>Glaucium leiocarpum</i>	Boynuzlu gelincik	Flowers, leaves	Diarrhea; headache	Decoction; wrapped with tobacco and smoked (external administration)	(Tuzlacı & Doğan, 2010)
<i>Glaucium grandiflorum</i>	Sarı gelincik	Fruits	Blood cleansing; eye ailments	Boiled and taken orally	(Ozsoy et al., 2018)
<i>Glaucium flavum</i>	Sarı haşhaş	Aerial parts	Wart treatment	Crushed and applied directly to the skin	(Emre et al., 2021)
<i>Glaucium corniculatum</i>	Kızıl gelincik	Fruit, root, whole plant	Eye disorders; burns; conjunctivitis; headache	Poultice; boiled aqueous preparation	(Bozkurt et al., 2022)
<i>Glaucium corniculatum</i>	Kızıl gelincik	Leaves	Food use	Cooked by frying with onion	(Eşen, 2008)
<i>Glaucium corniculatum</i>	Kızıl gelincik	Flowers	Stress relief; sedative use	Infusion	(Erucar et al., 2023)
<i>Glaucium cappadocicum</i>	Kapadokya haşhaşı	Whole plant	Antioxidant; antimicrobial	Ethanol extract; topical use	(Altın & Köksal, 2024)

6. Conclusion and Future Perspectives

The observed variability across biological assays highlights the intrinsic complexity of plant secondary metabolism. The pharmacological performance of plant extracts is strongly dependent on both the qualitative and quantitative composition of secondary metabolites, which are in turn shaped by multiple factors such as geographical origin, environmental conditions, soil characteristics, altitude, and plant developmental stage (Jain et al., 2019; Özay & Pehlivan, 2024). This inherent variability underscores the necessity of strategically designed extraction and fractionation approaches to selectively enrich bioactive constituents relevant to specific pharmacological targets.

Despite increasing research interest, the available evidence indicates that a substantial proportion of *Glaucium* species remain insufficiently explored from both phytochemical and pharmacological perspectives, with *G. flavum* clearly representing the most extensively investigated taxon to date. Systematic characterization of phytochemical profiles across the

genus is therefore essential to clarify chemotaxonomic relationships, elucidate intra- and interspecific chemical variability, and identify novel bioactive compounds with therapeutic relevance.

In agreement with extract-based findings, studies focusing on isolated isoquinoline alkaloids from *Glaucium* species have demonstrated that compounds such as glaucine, protopine, and sanguinarine often exhibit stronger and more selective inhibitory effects against acetylcholinesterase and butyrylcholinesterase than corresponding crude extracts. These observations highlight the importance of alkaloid scaffolds as key contributors to the neuropharmacological potential of the genus. Furthermore, the experimentally observed high antioxidant activities in certain *Glaucium* species indicate that this genus may possess protective potential against oxidative stress-induced cellular damage.

Taken together, these findings position *Glaucium* species as promising sources of multifunctional phytochemicals that integrate neuroprotective, antioxidant, anti-inflammatory, antimicrobial, and anticancer-related activities. Although most current evidence is derived from in vitro systems and rodent models, the convergence of ethnopharmacological relevance, phytochemical richness, and experimentally validated bioactivities warrants deeper pharmacological, toxicological, and mechanistic investigations. Future studies integrating advanced metabolomic profiling, target-based assays, and well-designed in vivo models will be critical to translating the traditional and experimental knowledge of *Glaucium* species into scientifically grounded pharmacological applications.

Authorship Contribution Statement

The author is solely responsible for the conceptualization, methodology, data collection, analysis, and manuscript preparation.

Conflict of Interest

The author declares no conflict of interest.

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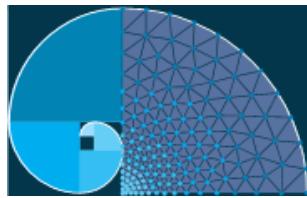
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The Impact of Rural to Urban Youth Migration on Labor Availability for Banana Production in Janale District, Somalia

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Abstract

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Agriculture is central to Somalia's economy and food security, yet it is increasingly constrained by rural-urban youth migration. This study examines the effects of youth migration on agricultural labor availability and banana farming in Janale District, a historically important banana-producing area along the Shabelle River. Using a descriptive research design, primary data were collected through structured questionnaires administered to 96 farming households, focusing on migration patterns, labor availability, youth participation in banana production, and perceived impacts on productivity and output. The findings show that youth migration is widespread and increasingly normalized, driven mainly by insecurity, access to humanitarian assistance, and limited rural education and employment opportunities. These dynamics have substantially reduced agricultural labor availability, particularly among young workers, resulting in low youth involvement in banana farming and widespread perceptions of declining productivity and banana output. Despite these constraints, only a minority of farmers have adopted labor-saving technologies or alternative practices, indicating limited adaptive capacity. The study demonstrates that youth migration has become a critical constraint on banana production in Janale District, with significant implications for rural livelihoods and food security and underscores the need for integrated policies that strengthen rural security, support agricultural investment, and create viable opportunities for youth within agriculture.

1. Introduction

Agriculture is the backbone of Somalia's economy, providing employment for a large part of the population and contributing significantly to the country's gross domestic product (GDP). In Somalia, agriculture sector is about 45.8% of Somalia's labor force aged 15 years and above is engaged in agriculture, while herding, fishing, and other agriculture-related activities account for 9.4%, 4%, and 7.2% of employment, respectively. Additionally, agriculture contributes approximately 61% of the country's GDP, making it a vital sector for economic

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growth and food security. Recent evidence from Somalia confirms that agricultural performance is closely linked to labor dynamics and broader economic outcomes, underscoring the sector's vulnerability to labor shortages (Osman et al., 2025a).

Among the key agricultural activities in Somalia, banana farming has historically played an important role, especially in the southern regions. Janale District, along the Shabelle River, was once a major center for banana production, benefiting from fertile soils and a favorable climate (Mohamed et al., 2025). In the 1990s, Somalia cultivated nearly 11,000 hectares of bananas, making it one of Africa's leading exporters (Food and Agriculture Organization of the United Nations [FAO], 2020). Rural-to-urban youth migration has been widely documented as a major contributor to agricultural labor shortages in sub-Saharan Africa, reducing farm productivity and threatening rural livelihoods (Osman et al., 2025b). However, decades of conflict and instability have caused a severe decline in banana cultivation. By 2020, the cultivated area had shrunk to just 1,388 hectares, and total production had dropped to 23,684 tons, a small fraction of global output (FAO, 2025). One of the primary reasons for this decline is the ongoing rural-to-urban migration of youth, which has significantly reduced the availability of agricultural labor in rural areas like Janale. Young people are leaving farming communities in search of better job opportunities, education, and living conditions in urban centers. This migration trend has created labor shortages, particularly in labor-intensive sectors such as banana farming. The shortage of young, able laborers has affected farm productivity and the incomes of farming households, as older farmers struggle to meet cultivation and harvest demands. Although several studies have explored youth migration in Somalia (Yusuf et al., 2019; Griffith et al., 2023; Osman & Abebe, 2023; Hoffmann et al., 2022), there is limited research focusing specifically on how this migration affects the availability of agricultural labor and banana production in Janale.

The objectives of this study are to examine the socio-economic and cultural characteristics of the study participants in Janale District, to investigate the impact of rural-to-urban youth migration on the availability of agricultural labor in the banana farming sector, to analyze the effects of reduced labor availability on banana farm productivity and household incomes, and to propose policy recommendations aimed at addressing labor shortages in banana production resulting from youth migration.

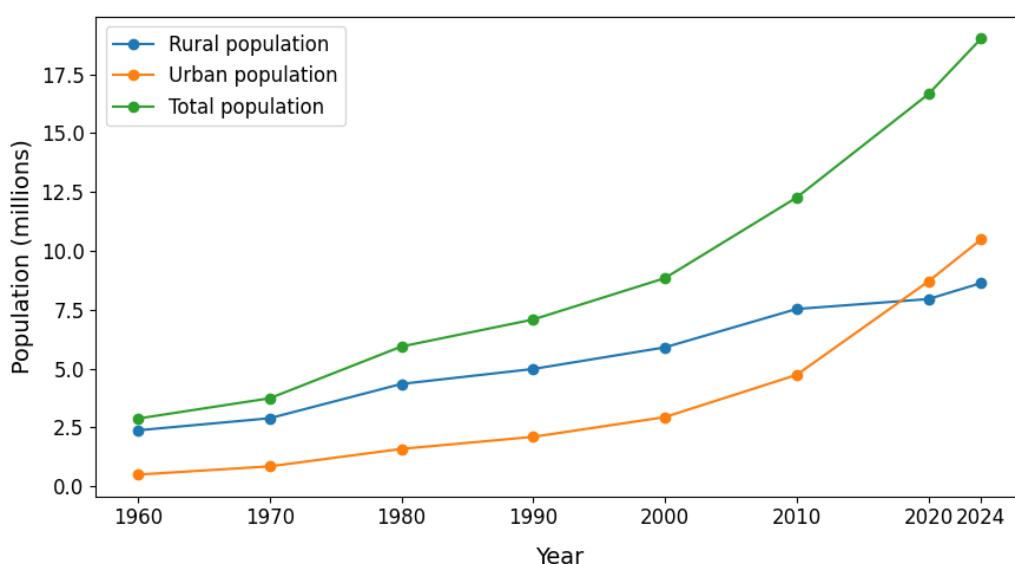


Figure 1. Rural and urban population trends in Somalia, 1960–2024 (World Bank, 2024)

Figure 1 depicts the historical distribution of Somalia's rural and urban populations from 1960 to 2024, highlighting a sustained decline in the rural population alongside rapid urban growth.

This long-term demographic shift reflects intensified rural-to-urban migration, particularly among youth, which has progressively reduced the availability of agricultural labor in rural areas. The acceleration of urbanization after the 1990s coincides with periods of economic disruption and conflict, contributing to labor shortages in agriculture and undermining productivity in labor-intensive sectors such as banana farming. These population dynamics align with trends reported in the World Bank's World Development Indicators, which document the structural implications of urbanization for rural livelihoods and agricultural systems in Somalia (World Bank, 2024).

Table 1 summarizes changes in banana production and harvested area in Somalia between 1961 and 2022, illustrating significant fluctuations in output and cultivated land over the period. The production and harvested area data presented in Table 1 are derived from FAO (2023). When interpreted alongside the population trends shown in Figure 1, these fluctuations reveal a strong structural linkage between demographic change and the long-term decline of banana cultivation. During the 1960s and early 1970s, when Somalia's population was overwhelmingly rural, banana production expanded markedly, supported by abundant agricultural labor and the labor-intensive nature of plantation farming in riverine areas such as the Shabelle Valley. From the 1980s onward, however, banana output and harvested area became increasingly unstable and ultimately declined, coinciding with a steady reduction in the rural population share and accelerating urbanization. Although temporary production recoveries were observed around 1990, these gains were not sustained, as continued rural-to-urban migration, youth out-migration, and conflict-induced displacement progressively constrained labor availability. By the 2000s and into the 2020s, when the rural and urban populations approached parity, banana production had fallen to historically low levels, indicating that labor shortages—rather than agro-ecological limitations alone—had become a binding constraint on the sector.

Table 1. Banana production and harvested area in Somalia, 1961–2022 (FAO, 2023)

Year	Area harvested (Ha)	Total production (Ton)	Change (%)
1961	1,100	98,000	-
1970	4,900	140,300	41.16
1980	2,600	60,400	-56.90
1990	6,200	110,000	82.11
2000	2,300	42,000	-61.81
2022	1,384	23,650	-43.68

2. Literature Review

The existing literature provides a multifaceted scholarly foundation for examining the relationship between rural-urban youth migration and agricultural labor dynamics in sub-Saharan Africa. Collectively, prior studies establish key conceptual frameworks for understanding migration decision-making, document structural tensions between demographic change and labor-intensive agricultural systems, and highlight persistent gaps in context-specific evidence for fragile and conflict-affected settings such as Somalia. A conceptual synthesis of this literature reveals three overarching themes: the complexity of economic, social, and environmental drivers of youth migration; the differentiated and often uneven impacts of migration on agricultural productivity and rural livelihoods; and a persistent policy divide between strategies aimed at retaining youth in agriculture and those promoting structural transformation through urbanization and labor reallocation.

2.1. The multidimensional drivers of youth migration: Beyond simple economics

The literature consistently frames rural-urban migration not as a singular event but as a dynamic, aspirational process. While foundational economic push-pull factors are affirmed— inadequate rural employment (Mbah et al., 2016), search for education (Mbah et al., 2016; Zakari et al., 2025), and lack of infrastructure (Rufai et al., 2021)—the abstracts complicate this narrative. Eckert et al. (2019) offer a crucial corrective to the presumed youth disinterest in agriculture, finding high interest in farming among Kenyan youth, suggesting migration is often a pragmatic choice amidst structural constraints, not a rejection of rural life. This is echoed in the work of Assan and Kharisma (2019), who highlight how neoliberal policies shape labor-seeking behavior within national boundaries. Furthermore, de Brauw et al. (2014) note the paradox of persistently low migration rates in some African contexts despite potential gains, hinting at invisible barriers—perhaps social, informational, or risk-related—highly relevant to a post-conflict setting like Somalia. This conceptual lens warns against reducing migration causation in Janale District to mere poverty, urging an investigation into the interplay of aspirations, perceived opportunity, and structural limitations.

2.2. The dual impact on agricultural labor: Depletion and restructuring

The most direct conceptual link to the article's focus is the documented effect of youth outmigration on farm labor. Multiple studies confirm a reduction in the agricultural labor force, leading to increased labor costs and lower productivity (Mbah et al., 2016; Zakari et al., 2025; Paudel et al., 2025). This establishes a clear, negative causal pathway for banana production in Janale. However, the literature also introduces moderating variables and countervailing effects. Paudel et al. (2025) and Langill et al. (2023) introduce the critical concept of intra-household dynamics and adaptation strategies. Their work suggests that the impact is not uniform but mediated by factors such as household composition (whose labor is lost), the use of remittances for farm investment, and adoption of labor-saving technologies or cooperative models. Langill et al. (2023) further highlight the potential for gendered restructuring of agricultural roles, a dimension often overlooked. This shifts the conceptual understanding from a simple labor drain to a complex process of agrarian change, in which households may adapt through mechanization, crop substitution, or task reallocation, potentially transforming the social relations of banana production.

2.3. The policy dilemma between rural retention and economic diversification

A central tension in the literature concerns divergent policy prescriptions for managing rural-urban youth migration. One strand, represented by Mbah et al. (2016) and Zakari et al. (2025), emphasizes rural retention strategies, advocating investments in physical and social infrastructure—such as roads, electricity, schools, and health facilities—alongside agricultural incentives including access to credit and production inputs to enhance the attractiveness of farming. This perspective aligns closely with efforts to mitigate the “push” factors driving youth out of rural areas. In contrast, another strand of the literature, exemplified by Rufai et al. (2021) and Bandiera et al. (2022), conceptualizes migration as an integral component of economic development and prioritizes improving labor market outcomes for migrants and receiving economies. Policy recommendations within this framework stress rural economic diversification, skills development, and the expansion of salaried employment opportunities, envisioning development pathways in which rural youth contribute to economic growth through higher-productivity and more diversified sectors rather than remaining exclusively in agriculture. This divide reflects a broader debate in the literature over whether policy should focus on retaining youth in agriculture or supporting structural transformation in which youth mobility contributes to wider economic development, despite short-term pressures on labor-intensive sectors such as banana farming.

2.4. Synthesis of existing evidence and research gaps in the Janale District context

The reviewed literature offers a robust conceptual foundation but also reveals a significant contextual gap. Most existing studies focus on West and East African settings, with limited attention to Somalia or other post-conflict contexts. The distinctive combination of clan-based land tenure systems, prolonged conflict, weak formal institutions, and the central economic importance of banana exports in districts such as Janale creates an analytical landscape that is not adequately captured in the current literature. Moreover, although remittances are acknowledged as an important outcome of migration (Adu Gyamfi et al., 2021; Madondo & Dhobha, 2025), their specific role in financing agricultural investment—particularly in perennial and labor-intensive crops such as bananas—remains insufficiently examined. In addition, Dessalegn et al. (2023) explicitly highlight a prevailing research bias toward migration drivers rather than its impacts, especially on sending communities, underscoring a critical gap that this study seeks to address.

In conclusion, these gaps frame the relevance of the Janale District case. The existing literature conceptualizes youth migration as a rational, aspiration-driven response to structural rural constraints, while also indicating its potentially adverse—yet contextually mediated—effects on agricultural labor availability. This study therefore navigates the tension between documenting migration-induced labor pressures on banana production and examining the adaptive strategies employed by rural households. By situating these dynamics within the unresolved policy debate between agricultural labor retention and broader rural transformation, the study advances a nuanced analysis that moves beyond simplistic narratives of rural decline.

3. Materials and Methods

3.1. Study area and population

The study was conducted in Janale District and surrounding villages in the Lower Shabelle region of Somalia, an area historically recognized for intensive banana production. The total estimated population of Janale and its neighboring villages is approximately 8,000, with a substantial proportion of residents engaged in agricultural activities, particularly banana farming.

Primary data were collected through structured questionnaires administered to individuals involved in agricultural production in Janale town and nearby villages. The survey focused specifically on farming households, with emphasis on assessing the impact of rural-to-urban youth migration on labor availability in banana production. In addition to primary field data, the study also utilized secondary sources, including reports from relevant institutions, peer-reviewed journal articles, theses, statistical databases, and other related publications, to contextualize and support the empirical findings.

The survey sample size was determined using the proportional sample size formula proposed by Newbold et al. (2019). To obtain a conservative and sufficiently large sample, the population proportion was set at $p=0.50$, which is standard practice when the true proportion is unknown. The calculation was based on a 95% confidence level and a margin of error of 10%. The sample size was computed using the following formula:

$$n = \frac{Np(1-p)}{(N-1)\sigma_p^2 + p(1-p)}$$

where

n is the required sample size;

N is the total population of Janale District;

p is the estimated population proportion (set to 0.50 to maximize sample size);

σ_p^2 is the variance of the estimated proportion, defined as $\sigma_p^2 = \frac{e^2}{z^2}$ as

e is the margin of error; and

z is the standard normal value corresponding to the selected confidence level.

Using this formula, the required sample size was calculated as 96 respondents. The collected data were analyzed using descriptive statistical techniques to assess the relationship between rural-to-urban youth migration and the availability of agricultural labor in banana production. The results provide empirical insight into the key labor-related challenges facing the sector and inform policy discussions aimed at enhancing agricultural productivity and rural livelihoods.

3.2. Instrument development and pretesting

Data were collected using a structured questionnaire developed based on relevant literature addressing rural-urban migration and agricultural labor dynamics, drawing in particular on prior studies by Yusuf et al. (2019), Griffith et al. (2023), Osman and Abebe (2023), as well as reports published by the FAO (2020).

3.3. Questionnaire structure and administration

The questionnaire was organized into four main sections covering socio-demographic characteristics, migration experiences and drivers, agricultural labor availability, and perceptions of productivity and sectoral change. The instrument was administered in Af-Somali, the local language, and subsequently translated into English for analysis. To ensure linguistic accuracy and conceptual equivalence, a back-translation procedure was employed.

3.4. Ethical considerations

Ethical approval was obtained from the Institutional Review Board of Zamzam University of Science and Technology (No. 2024-1). Participation was voluntary, and participants were informed of the study's purpose, their right to withdraw, and measures ensuring confidentiality and anonymity. Informed verbal consent was obtained prior to participation. No personal identifiers were collected, and all data were used solely for academic research in accordance with the Declaration of Helsinki.

4. Results

4.1. Demographic and socio-economic characteristics

Table 2 shows that the respondent population is predominantly young, with 57.4% aged 30 years or younger, indicating a strong representation of economically active age groups. The 21–25 age cohort constitutes the largest share (24.0%), while respondents aged 51 years and above are minimally represented (4.2%). The gender distribution is relatively balanced, with a slight male majority (55.2%). Most respondents are married (77.1%), reflecting prevailing socio-cultural norms and suggesting household responsibilities that may shape labor participation, household decision-making, and livelihood strategies.

Table 2. Demographic characteristics of respondents

Age Group	Frequency	Percentage
<20	18	18.8

21-25	23	24.0
26-30	14	14.6
31-35	13	13.5
36-40	7	7.3
41-45	9	9.4
46-50	8	8.3
>51	4	4.2
Total	96	100.0
Sex		
Male	53	55.2
Female	43	44.8
Total	96	100.0
Marital Status		
Single	15	15.6
Married	74	77.1
Divorced	7	7.3
Total	96	100.0

Table 3 indicates that the majority of respondents are economically active, with high employment levels dominated by agriculture, while the prevalence of family-based living arrangements underscores the continued importance of extended households in shaping labor allocation and livelihood strategies in rural areas.

Table 3. Educational status, employment, and family living arrangements of respondents

Currently studying	Frequency	Percentage
Yes	18	18.8
No	78	81.3
Total	96	100.0
Currently employed	Frequency	Percentage
Yes	78	81.3
No	18	18.8
Total	96	100.0
If yes, what job	Frequency	Percentage
Farmer	34	35.4
Shopkeeper	10	10.4
Transporter	12	12.5
Tractor driver	12	12.5
Rural construction worker	14	14.6
Fertilizer distributor	3	3.1
Student	11	11.5
Total	96	100.0
Family living	Frequency	Percentage
Yes	83	86.5
No	13	13.5
Total	96	100.0

Table 4 presents household characteristics, landholding sizes, and income diversification patterns, showing that most households are medium-sized with generally moderate landholdings, while the high reliance on non-agricultural income sources—particularly

remittances – underscores income diversification as a key livelihood strategy for coping with agricultural and labor-related constraints.

Table 4. Household characteristics, landholding size, and income diversification

Family size	Frequency	Percentage
Three-four	17	17.7
Five-six	35	36.5
Seven-eight	26	27.1
Nine-ten	14	14.6
Eleven and above	4	4.2
Total	96	100.0
Landholding size	Frequency	Percentage
Less than 50	4	4.2
51-100	16	16.7
101-150	38	39.6
151-200	21	21.9
200-250	13	13.5
251-300	1	1.0
301 above	3	3.1
Total	96	100.0
Source of income outside of agriculture	Frequency	Percentage
Yes	70	72.9
No	26	27.1
Total	96	100.0
Source of income outside agriculture	Frequency	Percentage
Construction	2	2.1
Security guards	13	14.0
Driver	18	19.0
Tailor	8	8.3
Warehouse worker	4	4.2
Remittance	30	31.0
Teaching	21	22.0
Total	96	100.0

4.2. Youth migration patterns and drivers

Table 5 shows that youth migration is widespread in the study area, with 70.8% of households reporting direct experience and 84.3% indicating rural-urban migration of at least one household member, suggesting that migration has become a common and increasingly normalized household strategy. The primary drivers include lack of security, access to aid and financial support from NGOs, education, and employment opportunities, while lifestyle-related motivations play a comparatively minor role, a pattern that aligns with the observed shift toward income diversification and reduced reliance on agriculture.

Table 5. Respondents' experience with youth migration, primary reasons for migration, and rural-urban household mobility

Respondents' experience with youth migration	Frequency	Percentage
Yes	68	70.8

No	28	29.2
Total	96	100.0
Primary reasons for youth migration from Janale		
Job opportunities	10	10.4
Education	13	13.5
Better quality of life	3	3.1
Lack of security	23	24.0
Access to aid and financial support from NGOs	19	19.8
Total	68	100.0
Household members' migration from rural to urban areas		
Yes	81	84.3
No	15	15.6
Total	96	100.0

4.3. Labor availability and impacts on banana farming

Table 6 summarizes farmers' perceptions of labor availability and youth involvement in banana farming, revealing that labor scarcity is a major constraint, as only 33.3% of respondents consider labor to be adequate while 66.7% report limited to no availability. Youth participation remains low, with 58.3% indicating no youth involvement, and most respondents perceive a significant or moderate decline in agricultural labor over time, largely attributed to youth migration. This assessment is reinforced by the fact that more than half of the farmers (56.3%) report direct experiences of labor shortages in banana farming linked to youth migration, underscoring its substantial impact on agricultural labor dynamics in Janale.

Table 6. Participants' perceptions of labor availability, youth involvement, and the effects of youth migration on banana farming in Janale

	Frequency	Percentage
Participants' perceptions on labor availability for banana farming in Janale		
Adequate	32	33.3
Limited	33	34.4
Insufficient	25	26.0
No labor force available	6	6.3
Total	96	100.0
Participant responses on youth involvement in banana farming in Janale		
Yes	40	41.7
No	56	58.3
Total	96	100.0
Effects of youth migration on agricultural labor availability for banana farming in Janale		
Significant decreased	43	44.8
Moderate decreased	33	34.4
No change	8	8.3
Increased	12	12.5
Total	96	100.0
Farmers' experiences of labor shortages in banana farming due to youth migration in Janale		
Yes	54	56.3
No	37	38.5
Not applicable	5	5.2

Total	96	100.0
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Table 7 indicates that youth migration has had a pronounced impact on agricultural labor availability and farm performance in banana farming in Janale. A substantial majority of respondents (78.2%) reported some degree of labor reduction, including 39.6% noting a slight reduction, 32.3% reporting a significant reduction, and 6.3% indicating that no labor force is available, while only 21.9% perceived no impact. Consistent with this pattern of labor decline, 64.6% of respondents reported a decrease in productivity, whereas 29.2% observed no change and a small minority (6.3%) perceived an increase in productivity, which may be attributed to the adoption of innovations, mechanization, or improved farm management practices. Despite the widespread labor constraints, only 40.6% of respondents reported adopting alternative farming practices such as labor-saving technologies, mechanization, or crop diversification, while the majority (59.4%) continue to rely on traditional farming practices. In terms of production outcomes, 83.3% of respondents reported a decline in banana output, including 44.8% indicating a slight decrease and 38.5% reporting a significant decrease, whereas only 16.7% perceived no impact. Overall, these findings demonstrate that youth migration has substantially reduced agricultural labor availability, negatively affected productivity and production levels, and exposed limited adaptive capacity within the banana farming sector to effectively mitigate labor shortages.

Table 7. Impact of youth migration on agricultural labor for banana farming in Janale

	Frequency	Percentage
Effects of youth migration on agricultural labor availability for banana farming in Janale		
Not impact	21	21.9
A slight reduction in labor	38	39.6
Significant reduction in labor	31	32.3
No labor available	6	6.3
Total	96	100.0
Impact of labor shortages on banana farming productivity in Janale		
Productivity has decreased	62	64.6
Productivity has stayed the same	28	29.2
Productivity has increased	6	6.3
Total	96	100.0
Participants' adoption of alternative farming practices due to labor shortages in banana farming		
Yes	39	40.6
No	57	59.4
Total	96	100.0
Perceptions of the impact of reduced agricultural labor on overall banana farming sector		
No impact	16	16.7
Slight decrease in production	43	44.8
Significant decrease in production	37	38.5
Total	96	100.0

5. Discussions

5.1. Demographic and socio-economic characteristics

The demographic structure of respondents reveals a rural labor system that is inherently vulnerable to youth migration. The dominance of young adults within the economically active age range reflects the central role of youth in agricultural labor, particularly in labor-intensive systems such as banana production. This same group, however, is also the most prone to migration under conditions of insecurity and limited economic opportunity. Long-term studies of Somali youth mobility show that migration has become a normalized livelihood strategy shaped by protracted conflict, generational expectations, and constrained rural futures, rather than an exceptional response to short-term shocks (Ciabbarri, 2024). In this context, the loss of youth labor directly weakens agricultural production capacity, making farms highly sensitive to even moderate levels of out-migration.

Socio-economic characteristics further explain why labor losses result in declining productivity rather than effective adaptation. Although most respondents reported being employed, agriculture remains the dominant livelihood, supplemented by fragmented rural non-farm activities that rarely generate sufficient capital for reinvestment. Similar findings from other African agrarian settings show that youth migration reduces family labor availability and undermines farm performance, particularly where production remains labor dependent (Zakari et al., 2025). The prevalence of medium-sized landholdings in Janale intensifies this vulnerability, as such farms rely heavily on household labor yet lack the resources to mechanize or hire workers. While remittances provide short-term household stability, evidence from Somalia indicates that displacement, insecurity, and institutional weakness limit their conversion into agricultural investment, reinforcing a cycle of labor loss and declining output (Osman & Abebe, 2023). Together, these dynamics explain why youth migration in Janale District contributes to persistent labor shortages and reduced banana productivity rather than structural transformation of the rural economy.

5.2. Youth migration patterns and drivers

The results show that youth migration in Janale District has become a normalized and widespread household strategy, rather than an occasional response to hardship. The high incidence of rural-urban migration indicates that mobility is now embedded in everyday livelihood decision-making, consistent with evidence from Somalia showing that migration has evolved into a routine response to prolonged insecurity, economic precarity, and institutional weakness (Griffith et al., 2023). In this context, migration is less an individual choice and more a collective household strategy aimed at managing risk and securing alternative income sources when agricultural livelihoods become increasingly uncertain.

The predominance of lack of security and access to NGO-related aid as migration drivers highlights the structural forces shaping youth mobility. Conflict and insecurity continue to push young people away from rural production systems, while the spatial concentration of humanitarian assistance in urban areas creates additional incentives for relocation (Ciabbarri, 2024; Griffith et al., 2023; Mbatha & Roodt, 2014). Education and employment opportunities further reinforce this pattern, reflecting broader African labor market conditions in which rural youth face limited access to stable, salaried employment and remain trapped in low-productivity agricultural work (Bandiera et al., 2022). As emphasized in the urbanisation literature, such migration does not automatically generate positive rural transformation; instead, where institutional capacity and rural-urban linkages are weak, it can intensify agricultural labor shortages and undermine productivity (Sakketa, 2023).

5.3. Labor availability and impacts on banana farming

The findings demonstrate that youth migration has translated into a systemic labor constraint in banana farming in Janale District. Most farmers perceive labor availability as limited or insufficient, alongside low youth involvement in banana production, indicating a structural

weakening of the local agricultural workforce. This pattern aligns with broader evidence from Africa showing that young people remain concentrated in informal, low-return agricultural work with few prospects for advancement, making exit from farming a rational response rather than an anomaly (Bandiera et al., 2022). In labor-intensive crops such as bananas, where timely operations are critical, even partial labor losses can disrupt production cycles. The widespread perception of declining labor availability over time, directly attributed to youth migration, suggests that Janale's banana sector is increasingly unable to reproduce its labor base.

The consequences of labor shortages extend beyond availability to productivity decline, reduced output, and limited adaptive response. Most respondents reported falling productivity and declining banana production, while only a minority adopted labor-saving technologies or alternative practices. This limited adaptation capacity reflects constraints highlighted in the literature on Somalia and sub-Saharan Africa, where insecurity, weak institutions, and lack of capital restrict farmers' ability to mechanize or reorganize production in response to labor shocks (Griffith et al., 2023; Sakketa, 2023). While a small share of farmers reported stable or improved productivity—likely linked to localized innovation or management improvements—these cases remain exceptions rather than evidence of sectoral transformation. Consistent with the livelihood constellation framework, migration may reduce household risk through remittances, but it simultaneously erodes agricultural labor and undermines productive capacity when supportive rural-urban linkages and investment channels are absent (Griffith et al., 2023). Overall, the results indicate that youth migration has become a critical driver of labor scarcity and production decline in Janale's banana farming, exposing the sector's vulnerability and limited resilience under conditions of prolonged rural out-migration.

6. Conclusions

This study demonstrates that rural-to-urban youth migration has substantial implications for banana farming in Janale District, Somalia. By documenting both the extent of household migration and its perceived effects on agricultural labor, productivity, and output, the study provides district-level empirical evidence of a growing constraint on local production systems. Youth migration in Janale is not an occasional response to hardship but an increasingly normalized household strategy, driven primarily by insecurity, uneven access to humanitarian assistance and services, and limited rural economic opportunities. As young and able workers exit agriculture, banana farming—an inherently labor-intensive activity—faces persistent labor scarcity. Most respondents reported reduced labor availability and linked this decline to lower farm performance and a sector-wide reduction in output, confirming that labor shortages have become a binding constraint on production rather than agro-ecological conditions alone.

The findings have important implications for Somalia's food security, rural development, and economic stability. Banana production has historically been a vital cash crop in southern riverine areas, and continued labor shortages risk further weakening one of the country's key agricultural value chains. At the household level, the growing reliance on remittances and non-agricultural income sources reflects a structural shift away from rural livelihoods, with long-term consequences for rural economies and the intergenerational transfer of farming knowledge and skills. While migration may reduce short-term risk through income diversification, it simultaneously undermines the productive capacity of banana farming where investment channels, mechanization, and supportive rural-urban linkages remain limited.

This study contributes to the literature by offering one of the first district-level empirical analyses linking youth migration to banana farming outcomes in Somalia, moving beyond general accounts of migration to demonstrate how labor loss directly reshapes agricultural

productivity and sector performance. To address these challenges, policy responses should prioritize strengthening rural security, designing rural development and aid interventions that support production zones, and creating attractive opportunities for youth within agriculture through skills training, access to inputs and credit, and targeted mechanization and labor-saving support. These measures are essential to stabilizing the rural labor force, safeguarding banana production, and promoting sustainable agricultural development in fragile settings.

Authorship Contribution Statement

Each author contributed significantly to the study. AIA: Statistical analysis, Data curation, Writing – review & editing. AHA: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. HMA: Data collection, Investigation, Validation, Writing – review & editing. HAO: Sampling design, Data curation, Visualization, Writing – review & editing. MAM: Interpretation of results, Validation, Writing – review & editing. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

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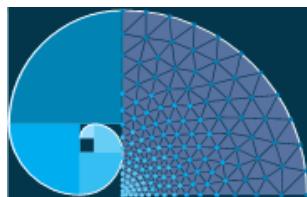
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Use of Rhizobacteria to Increase Tolerance to Salinity Stress in Tomato Growing

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Abstract

Salinity stress is a major and increasingly critical factor that negatively impacts plant growth, productivity, and overall agricultural sustainability. Addressing this challenge has therefore become a central focus in modern agricultural research. This study aimed to investigate the potential of beneficial rhizobacteria to enhance salt stress tolerance in tomato plants. For this purpose, six tomato varieties were used, and ultimately, only the most tolerant and the most sensitive varieties were selected for the study. Among the 19 bacterial isolates selected from laboratory stocks, three of the most successful isolates, identified *in vitro* under salt stress conditions, were chosen for further testing under *in vivo* conditions. The *in vivo* pot experiments were repeated twice. In the *in vivo* salt stress experiment, the selected bacterial isolates were applied to the salt-tolerant and salt-sensitive tomato varieties using seed bacterization and root immersion methods. It was determined that bacterial applications increased the fresh and dry weight of both shoots and roots compared to untreated control plants. Based on molecular identification and sequence analysis, these bacteria were identified as *Pantoea ananatis*, *Acinetobacter calcoaceticus*, and *Pantoea vagans*. These halophytic bacteria, known for their potential to enhance plant growth in saline environments, were evaluated as promising in terms of salt stress tolerance, plant growth promotion, and yield improvement, city and regional planning, forestry, agriculture and aquaculture.

1. Introduction

Tomato (*Solanum lycopersicum*) is a perennial vegetable belonging to the Solanaceae family. It is a versatile, popular, and one of the most important vegetable crops worldwide. Due to its unique nutritional value and widespread global production, it is recognized as a significant food source. Tomatoes can be grown in a variety of climates, including relatively cold regions, as they can be grown both outdoors and under protected conditions in many countries.

Like many crops, tomato cultivation is frequently exposed to various stresses that hinder plant growth and productivity. These stresses may be biotic, such as bacteria, viruses, fungi, and nematodes, or abiotic, including drought, salinity, water stress, temperature fluctuations, and mineral deficiencies. In the twenty-first century, several global challenges affecting agricultural systems have intensified, including global warming, water scarcity, environmental pollution, and the increasing salinization of agricultural lands and water

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resources. Environmental stressors such as strong winds, extreme temperatures, soil salinity, drought, and flooding have significantly affected the productivity and management of agricultural lands, with soil salinity representing one of the most severe constraints.

The climate crisis and improper land and water management practices are accelerating soil salinization, which affects more than 10% of the Earth's land surface (Food and Agriculture Organization [FAO], 2024). According to the FAO, approximately 20% of the world's irrigated agricultural lands are impacted by salinity, and nearly 10 million hectares of arable land are lost annually due to soil salinization worldwide (FAO, 2024).

Soil salinity develops through both natural and anthropogenic processes. Natural causes include the weathering of salt-bearing rocks, volcanic activity, and the deposition of sea salts transported through the atmosphere by wind. Anthropogenic salinization is primarily associated with improper irrigation practices and inadequate drainage systems. Additional contributing factors include low precipitation, high evaporation rates, and intensive agricultural activities. Moreover, climate change, excessive groundwater extraction, the use of low-quality irrigation water, intensive irrigation in semi-arid and arid regions, and insufficient soil leaching further exacerbate the salinization process. According to the first global assessment conducted in fifty years, 10.7% of global land is affected by salinity, a phenomenon increasingly intensified by climate change and human activities (FAO, 2024).

The prevention and management of salt stress in agricultural soils can be achieved through a range of integrated strategies, including the assessment of soil electrical conductivity (EC) to quantify salinity levels, the cultivation of salt-tolerant perennial crops, the selection of salt-resistant plant varieties, the use of salt-tolerant cover crops, and regular monitoring of irrigation water salinity. In addition, appropriate irrigation management and effective drainage systems can facilitate the removal of excess salts from the soil profile, while periodic soil leaching helps reduce salt accumulation within the root zone.

In recent years, increasing attention has been directed toward the use of non-pathogenic rhizobacteria, commonly referred to as Rhizobacteria (RB) or Plant Growth-Promoting Rhizobacteria (PGPR), due to their potential roles in the biological control of plant diseases, stimulation of plant growth, and enhancement of plant tolerance to abiotic stresses such as drought and salinity.

A growing body of research highlights the effectiveness of PGPR in mitigating salt stress and enhancing crop productivity. Egamberdieva et al. (2017) demonstrated that soil salinity significantly influences plant growth while emphasizing the dual role of rhizobacteria in growth promotion and biological control. In tomato cultivation, Medeiros and Bettoli (2021) reported that *Bacillus* spp. alleviated salt stress and concurrently suppressed Fusarium wilt under saline conditions. Recent studies have further identified PGPR as key salt stress attenuators contributing to sustainable agricultural (Al-Turki et al., 2023). Similarly, de Souza Ribeiro et al. (2023) showed that PGPR application mitigated salt-induced morphological stress in strawberry plants, while Yahyaoui et al. (2024) demonstrated significant improvements in growth and productivity of maize and tomato under saline soils following PGPR treatment. Collectively, these findings underscore the considerable potential of PGPR as an effective and sustainable strategy for enhancing crop resilience to salinity stress.

The aim of this study is to evaluate the potential of RB to mitigate salt stress in tomato plants. Specifically, the study investigates the effects of RB application under saline conditions by assessing key plant growth parameters, including leaf number and the fresh and dry weights of roots and shoots. Through this approach, the research seeks to contribute to the development of sustainable, biology-based strategies for managing salinity stress and enhancing tomato growth and productivity.

2. Materials and Method

2.1. Materials

2.1.1. Bacterial isolates used in the study

From the extensive RB collection (including epiphytic and endophytic strains) available in the Bacteriology Laboratory of the Dept. of Plant Protection at Ege University, 19 bacterial isolates were selected. These isolates were chosen based on previous projects where they had successfully promoted plant growth, as well as considering various *in vitro* test results and species differences. Selection criteria included key plant growth parameters such as ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase) activity, IAA (indole-3-acetic acid) production, phosphate solubilization activity, siderophore production, and other relevant traits.

2.1.2. Plant material used in this study

In this study, salt-tolerant and salt-sensitive tomato seeds were selected as test plants based on a preliminary trial conducted among widely cultivated commercial tomato varieties. For this purpose, seeds from six different tomato varieties (*Sakata F1 Jasmine 10*, *Sakata F1 Pink Pearl 13*, *Sakata Karasuta F-1 24*, *Sakata Beysin F-1 36*, *SC 2121*, and *Syngenta Torry 33*) were used.

2.1.3. Bacterial media and plant growth medium used in the study

In this study, King B (KB) and Tryptic Soy Agar (TSA) were used as culture media. KB was employed for culturing RB, while Nutrient Agar (NA) was used to evaluate growth kinetics to support production on a low-cost substrate (peat).

2.2. Method

2.2.1. *In vitro* tests

In this study, 19 bacterial isolates were evaluated for their tolerance to salinity. Bacterial cultures were obtained from 48-hour-old growth and streaked onto NA media supplemented with different concentrations of sodium chloride (NaCl) (0, 200, 400, 600, 850, 1000, 1500, and 2000 mM). The inoculated Petri dishes were incubated at 24 °C for 3–4 days, after which colony formation was assessed (Fischer et al., 2007; Ramadoss et al., 2013). Isolates capable of growth under high-salinity conditions were preserved at -80 °C in Nutrient Broth (NB) containing 10% NaCl and 30% glycerol for future use. Based on their growth performance, the most salt-tolerant isolates were selected for subsequent experiments.

In parallel, seeds of six tomato varieties were evaluated for germination percentage in accordance with International Seed Testing Association (ISTA) guidelines. Seeds were placed on filter papers moistened with NaCl solutions at concentrations of 0, 10, 20, 30, 40, 50, 100, 200, 1000, and 1500 mM. Prior to germination, seeds were surface-sterilized with 0.2% sodium hypochlorite (NaOCl), soaked in the respective salt solutions for 30 minutes, and then placed between double layers of filter paper moistened with the same solutions. Seeds placed on filter papers moistened with sterile distilled water served as the negative control. The experiment was arranged with 10 replicates per variety, each replicate consisting of 10 seeds. Seeds were incubated at 24 °C under high relative humidity (95%) for seven days, after which germination percentages were recorded.

Based on these results, one salt-sensitive and one salt-tolerant tomato variety were selected. The vigor index (VI) was determined under 0 and 40 mM NaCl treatments using the following formula:

$$VI = \text{Germination rate (\%)} \times (\text{Radicle length} + \text{Hypocotyl length})$$

To evaluate the effect of salt-tolerant bacterial isolates on germination performance under salinity stress, the most salt-sensitive tomato variety was used. Based on preliminary screening, the seven most salt-tolerant bacterial isolates were selected. Forty-eight-hour-old bacterial cultures were suspended in a 0.1% carboxymethyl cellulose (CMC) solution to prepare inocula. Surface-sterilized tomato seeds (0.2% NaOCl) were coated with the bacterial suspensions at a ratio of 5 g seeds per 5 mL suspension, shaken at 121 rpm for 30 minutes, and air-dried between filter papers at 24 °C for one hour (Sarma & Saikia, 2013).

Bacteria-coated seeds were placed in 9-cm Petri dishes containing double-layered sterile filter papers moistened with sterile water supplemented with 40 mM NaCl and incubated at 24 °C for seven days. The experiment was conducted with three replicates per bacterial isolate, each replicate consisting of 10 seeds. Seeds without bacterial inoculation or salt treatment served as the negative control, while seeds treated only with 40 mM NaCl served as the positive control. At the end of the incubation period, germination percentage was recorded, and the VI was calculated using the formula described above. The bacterial isolates that produced the highest germination rates and VI values were selected for subsequent *in vivo* experiments.

2.2.2. *In vivo* tests

Following preliminary trials, *in vivo* experiments were conducted using seeds from two tomato varieties identified as the most salt-sensitive and salt-tolerant, together with three bacterial isolates exhibiting the highest salinity tolerance. Bacterial inoculum was prepared from cultures grown on Nutrient Agar (NA) for 24–48 hours and suspended in a 0.1% CMC solution. Tomato seeds (5 g seeds per 5 mL suspension), previously surface-sterilized with 0.2% NaOCl, were coated with the bacterial suspension by shaking at 121 rpm for 30 minutes. The treated seeds were then air-dried between filter papers for 1 hour at 24 °C (Sarma & Saikia, 2013).

The bacterized seeds were sown in trays containing sterile peat. The experiment was arranged in a randomized complete block design with five replicates per treatment, each replicate consisting of one plant, and was conducted in a controlled growth chamber maintained at 24 °C with a 16 h light/8 h dark photoperiod. When seedlings reached the two-leaf stage, they were transplanted into 10 cm diameter pots containing sterile peat. The potting medium was homogeneously amended with 40 mM NaCl (11.6 g NaCl per 100 g peat).

After transplanting, seedlings were irrigated with a bacterial suspension containing the selected root bacterial isolates at a concentration of 10^8 CFU mL⁻¹ (50 mL per pot). For the negative control, seeds were coated only with 0.1% CMC, without bacterial inoculation or NaCl treatment. For the positive control, plants were grown in NaCl-amended peat (40 mM) without bacterial inoculation. Plants were monitored periodically for 6–8 weeks.

At the end of the experimental period, physiological and growth parameters, including plant height, number of compound leaves, and flowering status, were recorded. In addition, total plant biomass was determined by measuring fresh and dry weights for each replicate. Differences in biomass between positive and negative control treatments were quantified using the Abbott formula to calculate percentage effects. The *in vivo* experiments were repeated twice to ensure reproducibility and validation of results.

2.2.3. Molecular identification of salt-tolerant bacterial isolates

For molecular identification of the three bacterial isolates selected on the basis of *in vitro* and *in vivo* performance, including PGPR activity and salt stress tolerance, amplification of the 16S rRNA gene was performed using universal primer pairs 27F/1492R (Forward: 5'-AGA GTT TGA TCM TGG CTC AG-3'; Reverse: 5'-GGT TAC CTT GTT ACG ACT T-3') (Hodkinson & Lutzoni, 2009).

The PCR reaction mixture was prepared as a master mix, aliquoted into PCR tubes (20 μ L per reaction), and amplified using a thermal cycler under the following conditions: an initial denaturation at 95 °C for 5 minutes; 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 1 minute and a hold at 15 °C.

The amplified PCR products were resolved by electrophoresis on a 1.5% (w/v) agarose gel prepared in TAE buffer (0.5 M; Fermentas) and run at 80 V for 1 hour. DNA bands were visualized using a UV transilluminator, with amplicons observed at approximately 1460 bp. The PCR products were subsequently purified and submitted for sequencing, and the resulting sequences were compared with those available in the NCBI database to determine the taxonomic identity of the bacterial isolates.

2.2.4. Analysis of data

The experimental data were analyzed using one-way analysis of variance (one-way ANOVA). Differences among treatment means were assessed using Duncan's multiple range test at a 95% confidence level ($p \leq 0.05$).

3. Results

Within the scope of this study, bacterial isolates selected for salinity tolerance were initially evaluated under *in vitro* conditions and subsequently assessed under *in vivo* conditions.

3.1. *In vitro* results obtained from the determination of salt tolerance of bacterial isolates used in the study

In the study, a total of 19 bacterial isolates were cultured on NA medium to assess their salt tolerance at varying salt concentrations ranging from 0 to 2000 mM. After 72 hours of incubation at 24°C, the growth of the colonies was evaluated. Among the 19 bacterial isolates tested, 13 isolates exhibited good growth at a 1000 mM salt concentration. Among these, bacterial isolates 67, 121, 160, 213, 224, 300, and 302 demonstrated good growth even at a high salt concentration of 1500 mM (Figure 1).

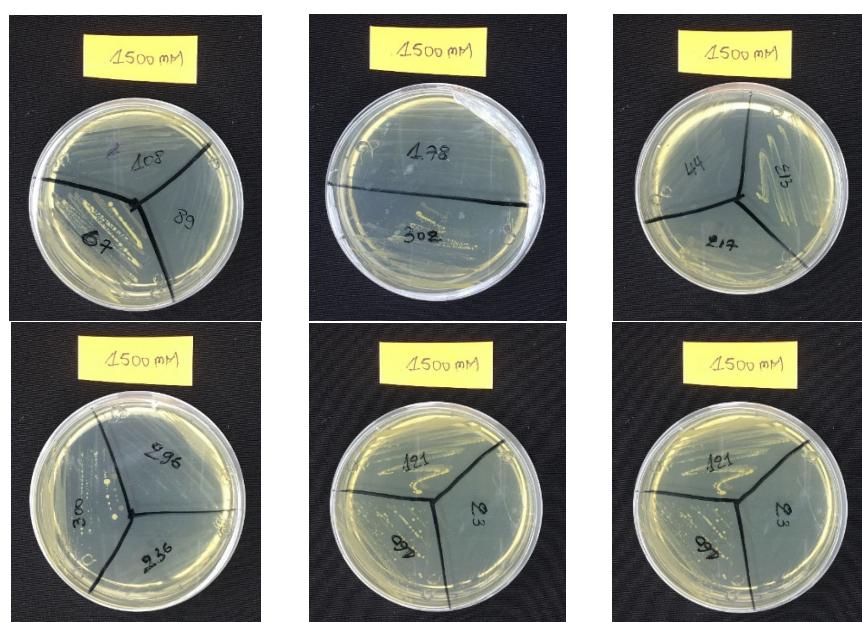


Figure 1. Growth of tested bacterial isolates on NA medium containing 1500 mM salt concentration

3.2. Results of the effects of different salt concentrations on germination rate (%) and VI of tomato varieties

The results obtained from testing the germination rate (%) of seeds from six different tomato varieties under various salt concentrations (0 mM, 10 mM, 20 mM, 30 mM, 40 mM) and later evaluating the VI using only the 0 mM and 40 mM doses are presented in Table 1.

Table 1. VI of tomato varieties at 40 mM salt concentration

Tomato cultivars	VI at different salt concentrations*	
	0 mM	40 mM
Syngenta 33	5143,33 bc**	9346,66 a**
Sakata 13	4224,66 bc	6223,33 b
Sakata 24	3464,66 bc	5564,33 bc
Sakata 36	3256,33 bcd	2333 d
Sakata 10	5688,60 bc	5456,66 bc
SC 21 21	2473,33 cd	2838 bcd

*Values represent the mean of four replicates, each consisting of ten seeds. Each experiment was conducted twice.

**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at $P \leq 0.05$.

3.3. Results obtained from the effects of salt-tolerant bacterial isolates on germination rate (%) and VI in salt-sensitive tomato cultivars

Based on the results obtained in section 3.2, it was determined that the tomato variety most sensitive to salt was SC2121. Before proceeding to *in vivo* tests, seeds of the most sensitive tomato variety were treated with bacterial isolates numbered 67, 121, 160, 213, 224, 300, and 302, which were identified as tolerant to salt. The effects of these bacteria on germination rate (%) and VI under a salt concentration of 40 mM were tested. The results obtained are presented in Table 2. According to the data in Table 2, in terms of germination rate and VI compared to the untreated positive control, isolate 302 was found to be the least affected by salt stress (Figure 2), followed by isolates 67 (Figure 3) and 121, respectively. Moreover, under conditions without salt stress, all seven bacterial isolates tested succeeded in increasing the VI compared to the untreated negative control (Table 2). Thus, among the bacterial isolates with the highest salt tolerance, isolates numbered 67, 121, and 302 were selected for *in vivo* tests.

Table 2. The effect of salt-tolerant bacterial isolates on the germination rate (%) and VI of the salt-sensitive tomato variety

Isolate No	Mean germination rate at different salt concentrations (%)*		Mean VI at different salt concentration*	
	0mM	40mM	0mM	40mM
K-	66,6 ab**	50 b**	2238,30 abc**	1078,3 bc**
67	70 ab	70 ab	3585,00 abc	2355 abc
121	73,3 ab	63,3 ab	3635,60 abc	2228,3 abc
160	83,3 a	63,3 ab	4883,00 a	2148,3 abc
213	73,3 ab	53,3 ab	3314,00 abc	1445,6 bc
224	73,3 ab	60 ab	3040,00 abc	785,6 c
300	73,3 ab	53,3 ab	3025,00 abc	764,6 c
302	80 ab	83,3 a	3708,30 abc	3801,6 ab

*Values represent the mean of four replicates, each with ten seeds. Each trial was repeated twice.

**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at $P \leq 0.05$.

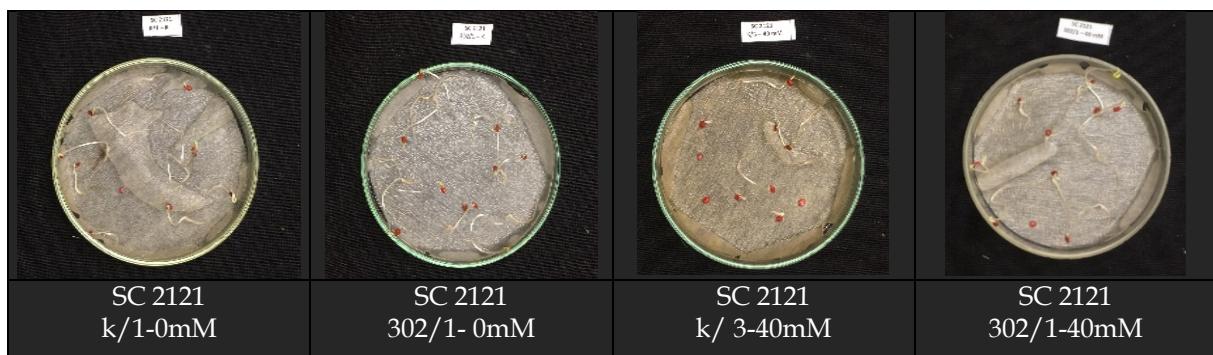


Figure 2. The effect of bacterial isolate number 302 on the germination of the SC2121 tomato variety in the absence and presence of salt stress (from left to right: Negative control, isolate 302 without salt stress, 40 mM salt stress (Positive Control), 40 mM salt stress + isolate 302)

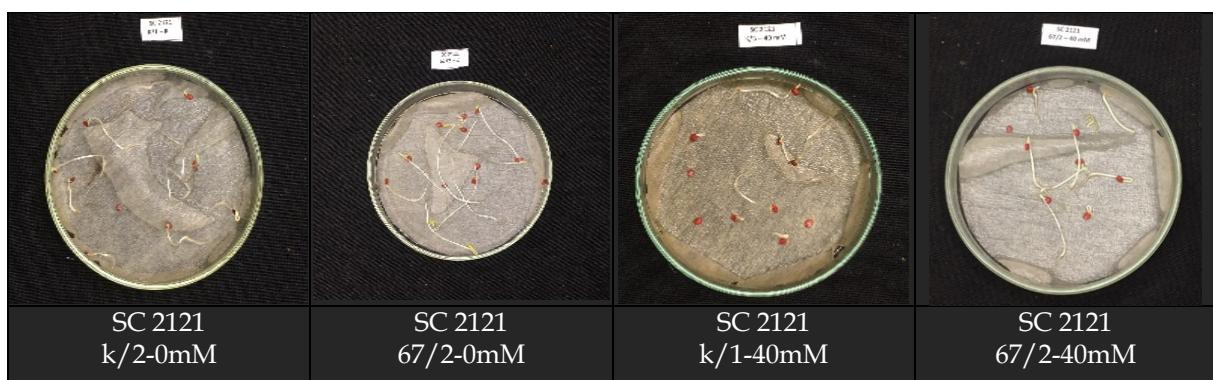


Figure 3. The effect of bacterial isolate number 67 on the germination of the SC2121 tomato variety in the absence and presence of salt stress (from left to right: Negative control, isolate 67 without salt stress, 40 mM salt stress (Positive Control), 40 mM salt stress + isolate 67)

3.4. *In vivo* test results

According to the results of the *in vitro* salt tolerance tests, *in vivo* tests were conducted in a plant growth chamber using two tomato varieties selected as the most sensitive and the most tolerant to salt, along with bacterial isolates numbered 67, 121, and 302, which were chosen from among the most salt-tolerant isolates. Tomato seeds treated via seed bacterization were transplanted into pots once they reached the two-leaf stage in seedling trays, in order to observe the effects of salt stress. The plants were monitored for growth under salt stress conditions for two months. The experiment was repeated twice, and the resulting shoot and root fresh and dry weight values are presented in Tables 3, 4, 5, and 6, respectively.

Table 3. The effect of bacterial treatments on shoot fresh weight in tomato varieties under salt stress

Tomato cultivars	Isolate no	I. Trial		II. Trial		Mean of two trials *	
		Mean shoot fresh weight (g/plant) *		Mean shoot fresh weight (g/plant) *		Mean of two trials *	
		0mM	40mM NaCl	0mM	40mM NaCl	0mM	40mM NaCl
Syngenta 33	K-	26,244 abcd**	24,092 abcd**	14,748 d**	30,608 abc**	20,496	27,350
	67	27,342 abcd	23,000 bcd	25,538 bc	32,815 ab	26,440	27,908
	121	28,444 abc	26,952 abcd	27,044 abc	33,784 a	27,744	30,368
	302	21,404 cd	26,712 abcd	26,114 bc	31,360 abc	23,759	29,036

SC 2121	K-	25,248 abcd	20,306 d	28,046 abc	30,282 abc	26,665	25,294
	67	29,146 ab	30,400 ab	25,026 c	32,364 ab	27,086	31,382
	121	27,034 abcd	30,442 a	28,328 abc	32,120 abc	27,681	31,281
	302	26,724 abcd	27,626 abcd	28,082 abc	28,382 abc	27,403	28,004

*Values represent the mean of five replicates, with one plant per replicate.

**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at $P \leq 0.05$.

Based on the mean shoot fresh weight values from the two trials, all bacterial treatments increased shoot fresh weight in both tomato varieties under both saline and non-saline conditions compared with the untreated negative control (Table 3).

Table 4. The effect of bacterial treatments on root fresh weight in tomato varieties under salt stress

Tomato cultivars	Isolate no	I. Trial		II. Trial		Mean of two trials*	
		Mean root fresh weight (g/plant) *		Mean root fresh weight (g/plant) *		Mean of two trials*	
		0mM	40mM NaCl	0mM	40mM NaCl	0mM	40mM NaCl
Syngenta 33	K-	1,160 ab **	1,536 ab**	1,526 de**	2,800 ab**	1,343	2,168
	67	1,532 ab	0,904 b	1,290 e	1,976 bcde	1,411	1,440
	121	1,252 ab	1,978 a	1,680 de	2,640 abc	1,466	2,309
	302	1,374 ab	2,062 a	3,112 a	3,036 a	2,243	2,549
SC 2121	K-	1,276 ab	0,862 b	1,832 cde	2,210 abcde	1,554	1,536
	67	1,246 ab	1,792 ab	1,936 bcde	1,932 bcde	1,591	1,862
	121	1,358 ab	1,492 ab	1,644 de	1,642 de	1,501	1,567
	302	1,462 ab	0,876 b	2,774 ab	2,314 abcd	2,118	1,595

*Values represent the mean of five replicates, with one plant per replicate.

**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at $P \leq 0.05$.

Based on the mean root fresh weight values obtained from the two trials, all bacterial treatments significantly increased root fresh weight in both tomato varieties under salt stress compared with the untreated negative control (Table 4). Under saline conditions, bacterial inoculation resulted in greater root biomass in the Syngenta 33 variety than in the SC2121 variety, with isolate 302 producing the most pronounced effect (Table 4).

Based on the mean shoot dry weight values from the two trials, bacterial treatments with isolates 121 and 302 increased shoot dry weight in the Syngenta 33 tomato variety under salt stress compared with the untreated negative control (Table 5). Overall, isolates 121 and 302 were the most effective bacterial treatments.

Table 5. The effect of bacterial treatments on shoot dry weight in tomato varieties under salt stress

Tomato cultivars	Isolate no	I. Trial		II. Trial		Mean of two trials*	
		Mean shoot dried weight (g/plant) *		Mean shoot dried weight (g/plant) *		Mean of two trials*	
		0mM	40mM NaCl	0mM	40mM NaCl	0mM	40mM NaCl
Syngenta 33	K-	2,922 abcd**	2,968 abcd**	1,846 d**	3,036 ab**	2,384	3,002
	67	3,402 abc	2,564 cde	2,206 cd	3,028 ab	2,804	2,796
	121	3,586 ab	2,934 abcd	2,556 abc	3,146 a	3,071	3,040
	302	2,426 de	3,730 a	2,590 abc	3,188 a	2,508	3,459
SC 2121	K-	2,746 bcde	2,014 e	2,392 bcd	2,376 bcd	2,569	2,195
	67	3,464 abc	3,404 abc	2,140 cd	2,622 abc	2,802	3,013
	121	3,242 abcd	3,356 abc	2,356 bcd	2,438 bcd	2,799	2,897
	302	3,078 abcd	2,766 bcde	1,992 cd	2,470 bcd	2,535	2,618

*Values represent the mean of five replicates, with one plant per replicate.

**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at $P \leq 0.05$.

Table 6. The effect of bacterial treatments on root dry weight in tomato varieties under salt stress

Tomato cultivars	Isolate no	I. Trial		II. Trial		Mean of two trials*	
		Mean root dried weight (g/plant) *		Mean root dried weight (g/plant) *		Mean of two trials*	
		0mM	40mM NaCl	0mM	40mM NaCl	0mM	40mM NaCl
Syngenta 33	K-	0,238 c**	0,250 c**	0,204 bc**	0,330 abc**	0,221	0,290
	67	0,304 c	0,202 c	0,148 c	0,272 abc	0,226	0,237
	121	0,346 bc	0,316 c	0,198 bc	0,328 abc	0,272	0,322
	302	0,224 c	1,060 a	0,376 ab	0,364 ab	0,300	0,712
SC 2121	K-	0,274 c	0,324 c	0,200 bc	0,250 bc	0,237	0,275
	67	0,376 bc	0,372 bc	0,198 bc	0,226 bc	0,287	0,299
	121	0,382 bc	0,366 bc	0,182 bc	0,196 bc	0,282	0,281
	302	0,294 c	0,670 b	0,290 abc	0,470 a	0,292	0,570

*Values represent the mean of five replicates, with one plant per replicate.

**Acc. to Duncan's multiple range test, values sharing the same letter are not significantly different at $P \leq 0.05$.

Based on the mean root dry weight values from the two trials, all bacterial treatments increased root dry weight under salt stress in both tomato varieties compared with the untreated negative control. Among the treatments, bacterial inoculation with isolate 302 resulted in the greatest increase in root dry weight (Table 6).

Overall, bacterial treatments were more effective in increasing total plant dry weight (root + shoot) under salt stress in the salt-tolerant tomato variety Syngenta 33 than in the salt-sensitive variety SC2121.

The results obtained from the *in vivo* trials were also evaluated in terms of total plant biomass. Figure 4 presents the mean total fresh weight (root + shoot) of plants grown under salt stress, while Figure 5 shows the corresponding mean total dry weight (root + shoot).

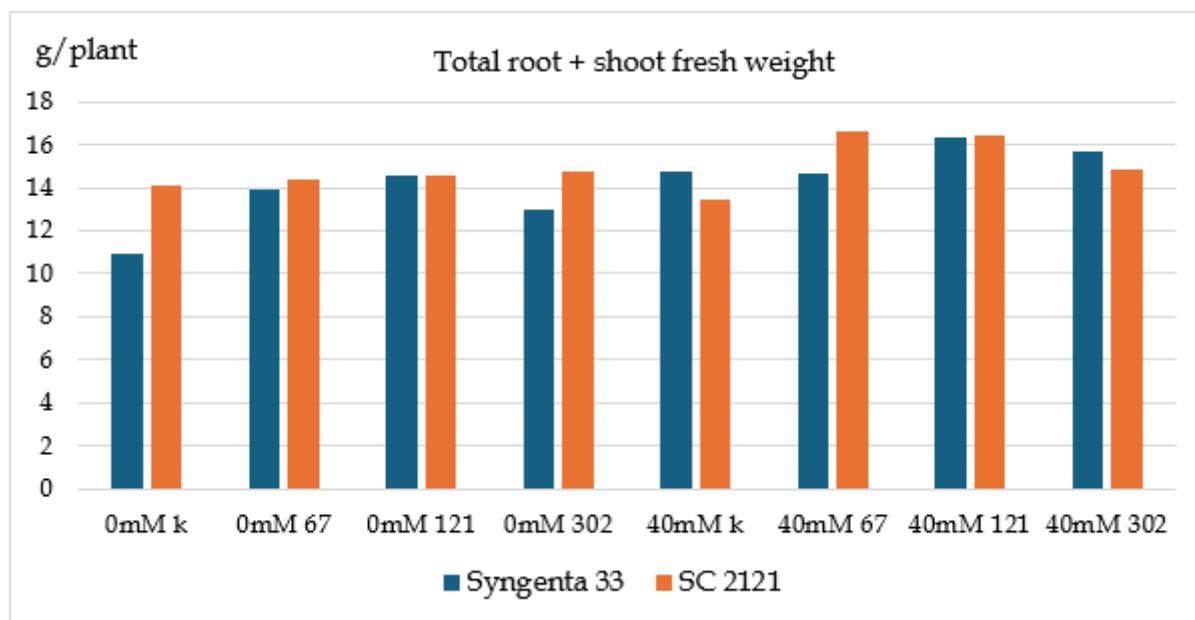


Figure 4. The effect of bacterial treatments on total plant fresh weight in the presence and absence of salt stress

As shown in Figure 4, bacterial applications exerted a more pronounced positive effect on total fresh plant weight (root + shoot) under salt stress than under non-saline conditions. In both tomato varieties exposed to salinity, all three bacterial treatments increased fresh plant weight.

Analysis of total dry plant weight under both saline and non-saline conditions (Figure 5) revealed that bacterial isolate 302 produced the greatest increase in dry biomass under 40 mM salt stress compared with the untreated control. This effect was followed by isolates 121 & 67.

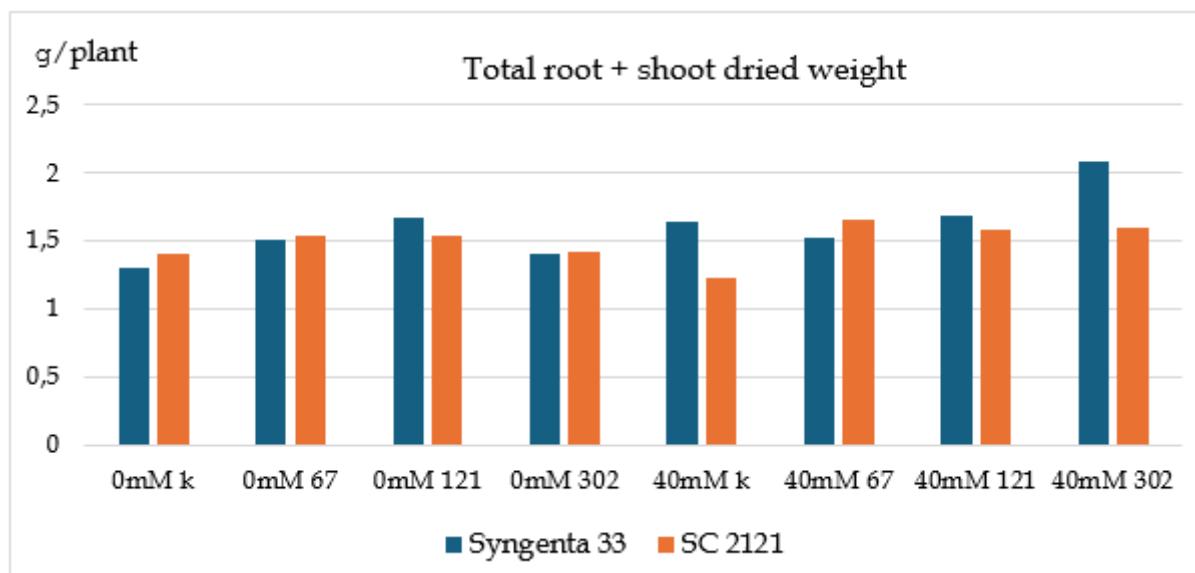


Figure 5. The effect of bacterial treatments on total plant dry weight in the presence and absence of salt stress

3.5. Molecular identification results of salt-tolerant bacterial isolates

In the molecular identification of the three bacterial isolates selected based on *in vitro* and *in vivo* trials (PGPR activity and salt stress), universal primer pairs designed for the 16S rRNA target region were used (Hodkinson and Lutzoni, 2009). The obtained PCR products were sent for sequencing, and the sequencing results were compared with information in the NCBI database to identify the bacterial isolates. As a result of the sequencing, isolate number 302 was found to be the most effective against salt stress in this study, followed by isolate number 121 and then isolate number 67 (Table 7).

Table 7. Sequence analysis of bacterial isolates effective against salt stress

Isolate code	Species / isolate no	NCBI accession number
FO67	<i>Pantoea ananatis</i> strain KD20/2	PQ009206
FO121	<i>Acinetobacter calcoaceticus</i> strain KD40/2	PQ009214
FO302	<i>Pantoea vagans</i> strain KD120/1	PQ009608

4. Discussion

Endophytic and rhizospheric bacteria play an increasingly important role in enhancing plant growth and resilience under adverse environmental conditions. Endophytes are non-pathogenic microorganisms that colonize internal plant tissues and often confer adaptive advantages to their hosts, particularly under biotic and abiotic stress (Vaishnav et al., 2018). Among abiotic stresses, soil salinity is one of the most severe constraints on plant growth and agricultural productivity worldwide. Consequently, sustainable, biology-based strategies—such as the use of PGPR—are gaining attention as effective alternatives to conventional breeding and genetic engineering approaches.

In the present study, salinity was selected as the primary abiotic stress factor, and bacterial isolates from laboratory stocks were evaluated for their capacity to mitigate salt stress in tomato plants under both *in vitro* and *in vivo* conditions. The isolates were initially screened based on key PGPR traits, including indole-3-acetic acid (IAA) production, siderophore secretion, phosphate solubilization, and 1-aminocyclopropane-1-carboxylate deaminase (ACCD⁺) activity. These traits are widely recognized as reliable indicators of bacterial potential to enhance plant growth and stress tolerance. The results confirmed that preliminary *in vitro* screening based on these parameters is an effective approach for selecting salt-tolerant bacterial candidates.

A salinity level of 40 mM NaCl, previously reported to adversely affect tomato growth (Sahab et al., 2021), was used for *in vivo* evaluation. The selected bacterial isolates demonstrated a strong ability to tolerate high salt concentrations in *in vitro* assays and significantly improved plant growth under 40 mM NaCl in pot experiments. Increases in fresh and dry biomass indicated that bacterial inoculation effectively mitigated the inhibitory effects of salinity, confirming the suitability of this stress level for evaluating bacterial-mediated tolerance in tomato.

One of the most critical mechanisms underlying bacterial-induced salt tolerance is ACC deaminase activity. Under salt stress, plants produce excessive ethylene, which restricts root elongation, accelerates senescence, and triggers programmed cell death. ACCD⁺ bacteria reduce stress-induced ethylene levels by degrading ACC, thereby promoting root growth and maintaining cellular viability (Choudhury et al., 2023). The bacterial isolates identified in this study—*Pantoea ananatis*, *Acinetobacter calcoaceticus*, and *Pantoea vagans*—exhibited strong ACCD⁺ activity, which likely contributed to their consistent performance in improving tomato growth under saline conditions. These findings align with previous reports demonstrating

that ACCD⁺ PGPR enhance biomass accumulation and delay stress-induced senescence through ethylene regulation.

In addition to ACC deaminase activity, IAA production and siderophore secretion were key traits associated with bacterial effectiveness in this study. High levels of IAA enhance root system development, increasing the plant's capacity for water and nutrient uptake under stress conditions. Siderophore production improves iron availability and may indirectly suppress pathogenic microorganisms, contributing to improved plant vigor. The strong expression of these traits among the selected isolates supports their role as reliable selection criteria for identifying effective PGPR under salinity stress.

Exopolysaccharide (EPS) production is another mechanism linked to bacterial-mediated salt tolerance. EPS can bind sodium ions, reduce ionic toxicity, enhance soil aggregation, and improve water retention in the rhizosphere. Previous studies have shown that EPS-producing bacteria promote plant survival and growth under saline conditions by acting as protective "stress molecules" (Tahmish & Naveen, 2023). The presence of effective *Pantoea* group isolates in the present study further supports the hypothesis that EPS production may have contributed to the observed improvements in plant biomass under salt stress.

The effectiveness of PGPR under saline conditions has been demonstrated across multiple crops. For example, *Bacillus velezensis* JB0319 enhanced lettuce growth, antioxidant enzyme activity, and osmotic balance under salt stress while also reshaping the rhizosphere microbial community (Bai et al., 2023). Similarly, PGPR applications alleviated NaCl-induced morphological and physiological damage in strawberry plants (Sahab et al., 2021). The positive effects observed in the present study using seed bacterization and root inoculation methods are consistent with these findings, highlighting the effectiveness of these application strategies for delivering beneficial bacteria into plant systems.

Increasing environmental challenges and global hunger have driven the development of stress-tolerant crops through genetic engineering and plant breeding. However, these approaches are time-consuming and difficult to implement on a large scale. As an alternative, sustainable agricultural practices involving beneficial bacteria have emerged as promising solutions. These bacteria enhance plant tolerance to salt stress by reducing salt uptake, notably through the regulation of specific ion transporters and by trapping ions within exopolysaccharide matrices (Bhat et al., 2020), which partly explains their observed effectiveness in this study.

Among the isolates identified, *Pantoea ananatis* emerged as a particularly promising halotolerant PGPR. Previous studies have shown that *P. ananatis* enhances plant growth, chlorophyll content, protein accumulation, and proline levels under salt stress (Lu et al., 2021). In agreement with these reports, the *P. ananatis* isolate identified in this study significantly promoted tomato growth under both saline and non-saline conditions, supported by strong ACCD⁺ activity, IAA production, and siderophore secretion. Likewise, the effectiveness of the *Acinetobacter* isolate is consistent with earlier studies demonstrating the capacity of *Acinetobacter* spp. to enhance plant growth and seed germination under high salinity (Patel et al., 2022).

Overall, the findings of this study demonstrate that PGPR possessing ACC deaminase activity, IAA production, siderophore secretion, phosphate solubilization, and EPS production can effectively mitigate the adverse effects of salinity stress in tomato plants. Compared with conventional breeding and genetic engineering approaches—which often require long development periods—the use of beneficial bacteria offers a rapid, sustainable, and climate-smart strategy for improving crop resilience. Importantly, this study confirms that systematic *in vitro* screening based on key PGPR traits provides a reliable framework for selecting effective bacterial candidates for *in vivo* application in salt-affected agricultural systems.

5. Conclusion

Salinity is one of the most pervasive abiotic stresses threatening global food security and environmental sustainability, with projections indicating that up to 50% of arable land may be affected by salinization by 2050. Salt accumulation degrades soil fertility, limits crop productivity, and disrupts ecosystem functioning. In this context, PGPR offer a promising, environmentally sustainable strategy to enhance plant performance under saline conditions while contributing to the remediation of salt-affected soils.

In this study, several beneficial bacterial isolates demonstrated a strong capacity to mitigate salt stress in tomato plants. Among them, *Pantoea vagans* isolate 302 produced the most pronounced increases in fresh and dry plant biomass in both salt-sensitive and salt-tolerant tomato cultivars grown under 40 mM NaCl. Similarly, *Acinetobacter calcoaceticus* isolate 121 and *Pantoea ananatis* isolate 67 also significantly enhanced plant growth. Notably, bacterial inoculation improved plant biomass even relative to non-saline controls, highlighting the robust plant growth-promoting potential of these isolates.

Genotypic differences among tomato cultivars further influenced plant responses to salinity, emphasizing the importance of selecting salt-tolerant varieties as part of integrated stress management strategies. In addition to alleviating abiotic stress, the PGPR strains evaluated here exhibit multifunctional traits, including potential roles in biological control of plant pathogens and insect pests, supporting their application within integrated pest management frameworks.

Overall, the use of plant growth-promoting bacteria represents a sustainable and climate-smart approach to enhancing crop resilience. Future research should focus on field validation and the development of effective bioformulations to facilitate large-scale agricultural application.

Authorship Contribution Statement

FD carried out the growth chamber and colonization studies, and performed the statistical analysis, sequence alignment, and drafted the manuscript. HÖ designed and coordinated the study. All authors have read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request.

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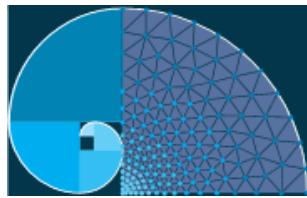
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A Novel Semi-Analytical Approach for High-Order Delay Differential Equations Based on History Functions: Application to Nonlinear Vibration of Delayed Systems

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Abstract

In this study, a novel semi-analytical approach based on the Perturbation-Iteration Algorithm is proposed for solving high-order delayed differential equations using history functions. By employing the method of steps to transform the delayed problem into a system of ordinary differential equations defined over sub-intervals, the proposed approach offers a systematic solution framework distinct from existing methods in the literature. Another significant contribution of this study is the development of an algorithmic procedure for determining the initial function that initiates the iteration process. By incorporating the history function and continuity conditions between consecutive intervals directly into the governing equations through matrix operations, this procedure enables the algorithm to generate smooth and high-precision solutions within each sub-interval. The proposed method is applied to the dynamic analysis of the delayed Mathieu and delayed damped Mathieu equations, which play a critical role in nonlinear vibration theory. Parametric investigations reveal that positive delay coefficients effectively suppress vibration amplitudes by introducing an artificial damping effect, while negative delay coefficients may counteract physical damping and drive the system toward instability; additionally, increases in excitation amplitude intensify oscillatory responses and slightly reduce convergence speed. The results demonstrate that the developed approach is an effective and reliable tool for modelling and analyzing complex engineering problems involving delay terms.

1. Introduction

Delay differential equations (DDEs) are essential mathematical tools for modelling dynamic processes in which the rate of change of a system depends not only on its current state but also on its past states. As described by Polyanin et al. (2023) and Ford (2025), phenomena involving "after-effect" and time delay, encountered in fields such as engineering, biology (Rihan, 2021), and economics, can fundamentally alter a system's dynamic behavior. Accurate modelling of delay terms is crucial for system stability in high-precision industrial applications, such as predicting chatter vibrations in milling processes (Yang et al., 2023), as also demonstrated in high-dimensional delay differential equation studies of crossflow-induced nonlinear vibrations in steam generator tubes (Sun et al., 2023).

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Obtaining analytical solutions for DDEs is inherently more challenging than for classical ordinary differential equations. This has led researchers to develop efficient numerical and semi-analytical methods. The literature presents various numerical techniques, including Variable Multistage Methods (VMM) for higher-order DDEs (Martín & García, 2002) and the Direct Adams-Moulton Method (DAM) for second-order problems (Seong et al., 2013). Kumar and Methi (2021) proposed efficient numerical algorithms for nonlinear DDEs, while Khuri and Sayfy (2017) developed a Green's function-based iterative approach for functional differential equations.

In addition to numerical methods, semi-analytical methods are often preferred for solving complex problems. Barde and Maan (2019) proposed the Natural Homotopy Analysis Method for nonlinear DDEs, Olvera et al. (2015) used the Enhanced Multistage Homotopy Perturbation Method (EMHPM), and Aziz and Amin (2016) used Haar wavelets to provide approximate solutions for delayed equations. Amirali (2023) established stability inequalities for linear nonhomogeneous Volterra delay integro-differential equations. Recently, Ohira and Ohira (2025) introduced new approaches involving the Fourier transform for solving DDEs.

In recent years, polynomial-based matrix methods have become prominent in solving complex differential equations due to their high accuracy and wide applicability. Çevik et al. (2025) provided a comprehensive review of polynomial matrix collocation methods in engineering applications. Additionally, Cayan et al. (2022) proposed effective solutions for linear and nonlinear engineering models using a Taylor-splitting collocation approach. In this context, Dağ and Biçer (2026) developed a new method for nonlinear DDEs using Boole polynomials. Previous work has also addressed the solution of pantograph-type delayed equations using orthoexponential polynomials (Bahşı et al., 2015), Adomian decomposition method (Alenazy, et al., 2022), Taylor polynomials (Yüzbaşı & Ismailov, 2018) and neural networks (Bahşı & Bahşı, 2025).

The Mathieu equation, a central focus of this study, plays a key role in the stability analysis of parametrically excited systems. In their study on generalizations of the Mathieu equation, Kovacic et al. (2018) highlighted the importance of stability charts for these equations. However, the analysis of Mathieu equations with time delay and damping still requires novel, high-precision solution methods, particularly due to the need to account for history functions (Blanco-Cocom et al., 2012).

The Perturbation-Iteration Algorithm (PIA) has been successfully applied to solve pantograph-type (variable delay qt) differential equations (Bahşı & Çevik, 2015). However, while the delay in pantograph-type equations is proportional, in many engineering problems the delay is a constant duration τ ($t - \tau$), and the system behaviour is determined by a history function $h(t)$. In such constant delay problems, it is necessary to divide the solution domain into sub-intervals and ensure continuity within each interval.

In this study, unlike existing methods in the literature, a novel semi-analytical algorithm based on the PIA is presented for high-order delay differential equations using history functions. The main innovation of the proposed method is the integration of the PIA with the "method of steps," which divides the solution domain into sub-intervals, and the inclusion of a systematic matrix-based procedure for determining initial functions that ensures continuity at each sub-interval transition. This approach aims to provide faster convergence and greater accuracy than methods currently available in the literature. Unlike existing step-based and semi-analytical methods that impose inter-interval continuity in a limited manner, the proposed approach introduces a systematic matrix-based continuity enforcement that simultaneously satisfies all derivative matching conditions, resulting in smoother, more robust, and faster-converging solutions for high-order delay differential equations.

The study is structured as follows: Section 2 outlines the mathematical framework of the method. Section 3 evaluates the accuracy of the proposed method by comparing it with

numerical methods from the literature. Section 4 applies the method to the dynamic analysis of the delayed Mathieu equation (DME) and delayed damped Mathieu equation (DDM), which are important in computational mechanics, to examine the effects of delay and damping parameters on system stability.

2. Methodology

This section presents the mathematical framework for solving delay differential equations using the PIA. The solution strategy is based on the “method of steps,” which transforms the delayed problem into a sequence of ordinary differential equations defined over sub-intervals. First, the general formulation of the problem and the domain decomposition strategy are introduced. Next, the derivation of the PIA(1,1) algorithm is detailed, followed by the procedure for determining the initial functions required to ensure continuity between successive intervals.

2.1. General formulation of delay differential equations based on history functions

The general form of the k -th order delay differential equation, which includes the delay term $u(t - \tau)$ or at least one of its derivatives is given in implicit form as:

$$F\left(u^{(k)}, \dots, u', u, u_{\tau}^{(k)}, \dots, u'_{\tau}, u_{\tau}, \varepsilon, t\right) = 0 \quad (1)$$

where $0 \leq t \leq b$, $u = u(t)$, $u_{\tau} = u(t - \tau)$ such that $\tau > 0$, and ε is the perturbation parameter. The conditions for this equation, where $\alpha_{1i}, \alpha_{2i}, \alpha_{3i} \in R$ for all i , $\beta \in R$ such that $a < \beta < b$, and $h(t)$ is defined in the interval $[-\tau, 0]$, are given as:

$$\sum_{j=0}^{k-1} \left(\alpha_{1i} u^{(j)}(0) + \alpha_{2i} u^{(j)}(\beta) + \alpha_{3i} u^{(j)}(b) \right) = \gamma_i, \quad i = 1, \dots, k-1 \quad (2)$$

and the history function is defined as:

$$u(t) = h(t), \quad -\tau \leq t \leq 0 \quad (3)$$

In the solution of Eq. (1) for the first sub-interval $[0, \tau]$, the delay term $u(t - \tau)$ is calculated using the history function $h(t)$. Specifically, for $t \in [0, \tau]$, the relationship is defined as $u(t - \tau) = h(t - \tau)$. Within this same sub-interval, any derivative of the delay term corresponds to the derivative of the history function of the same order. By substituting the history function or its derivatives into the corresponding delay terms, the solution over the interval $[0, \tau]$ is obtained using the proposed PIA algorithm. For the remaining sub-intervals of the domain $[0, b]$ where $t \geq \tau$, the history function is defined by the PIA solution obtained in the preceding sub-interval. Consequently, the global solution for the entire domain is constructed by combining the solutions derived from all sub-intervals.

2.2. Domain decomposition

The domain of the problem $[0, b]$ is divided into J sub-intervals as follows:

$$J = \left\lceil \frac{b-0}{\tau} \right\rceil \quad (4)$$

where $\lceil \cdot \rceil$ denotes the ceiling function. The domain $D = [0, b]$ is expressed as the union of sub-intervals D_j for $j = 1, 2, \dots, J$:

$$D = \bigcup_{j=1}^J D_j = \left(\bigcup_{j=1}^{J-1} [(j-1)\tau, j\tau] \right) \cup [(J-1)\tau, b] \quad (5)$$

The dependent variable $u(t)$ is defined as u_{D_j} in the sub-interval D_j . The equation is converted into a system of equations for each sub-interval:

$$F^{D_j}((u^{D_j})^{(k)}, \dots, (u^{D_j})', u^{D_j}, \varepsilon, t) = 0, \quad t \in D_j \quad (6)$$

For $j > 1$, the delay terms in the interval D_j are equal to the n -th iteration solution of the previous interval $u_n^{D_{j-1}}(t)$. The global n -th iteration solution $u_n(t)$ is defined as:

$$u_n(t) = \begin{cases} u_n^{D_1}(t) & , \quad t \in D_1 \\ u_n^{D_2}(t) & , \quad t \in D_2 \\ \vdots & \vdots \quad \vdots \\ u_n^{D_J}(t) & , \quad t \in D_J \end{cases} \quad (7)$$

2.3. PIA(1,1) algorithm

For the n -th iteration solution $u_n^{D_j}$ in the interval D_j , a direct expansion with a single correction term is applied:

$$(u^{D_j})_{n+1} = (u^{D_j})_n + \varepsilon \left(u_c^{D_j} \right)_n, \quad j = 1, 2, \dots, J \quad (8)$$

where $\left(u_c^{D_j} \right)_n$ is the n -th correction term. Substituting Eq. (8) into Eq. (6) and expanding in a Taylor series up to the first order derivative yields:

$$\begin{aligned} & F^{D_j} \left((u^{D_j})_n^{(k)}, \dots, (u^{D_j})_n, 0, t \right) \\ & + F_{(u^{D_j})}^{D_j} \left((u^{D_j})_n^{(k)}, \dots, (u^{D_j})_n, 0, t \right) \varepsilon \left(\left(u_c^{D_j} \right)_n \right)^{(k)} \\ & + F_{(u^{D_j})}' \left((u^{D_j})_n^{(k)}, \dots, (u^{D_j})_n, 0, t \right) \varepsilon \left(\left(u_c^{D_j} \right)_n \right)' \\ & + F_{u^{D_j}}^{D_j} \left((u^{D_j})_n^{(k)}, \dots, (u^{D_j})_n, 0, t \right) \varepsilon (u_c)_n^{D_j} \\ & + F_{\varepsilon}^{D_j} \left((u^{D_j})_n^{(k)}, \dots, (u^{D_j})_n, 0, t \right) \varepsilon = 0 \end{aligned} \quad (9)$$

This equation can be simplified as the PIA(1,1) algorithm:

$$F_{(u^{D_j})}^{D_j} \left(u_c^{D_j} \right)_n^{(k)} + \dots + F_{(u^{D_j})}' \left(u_c^{D_j} \right)_n' + F_{u^{D_j}}^{D_j} \left(u_c^{D_j} \right)_n = -F_{\varepsilon}^{D_j} - \frac{F^{D_j}}{\varepsilon} \quad (10)$$

2.4. Determination of initial functions

For $j > 1$, the initial function $u_0^{D_j}(t)$ for the interval D_j is determined using the PIA(1,1) solution obtained in the previous interval D_{j-1} , ensuring continuity at the boundary $(j-1)$. The continuity conditions are:

$$\left(u_0^{D_j} \right)^{(m)}((j-1)\tau) = \left(u_n^{D_{j-1}} \right)^{(m)}((j-1)\tau), \quad m = 0, 1, \dots, k-1 \quad (11)$$

The initial function is defined by a polynomial of degree $(k - 1)$, $P_{k-1}^{D_j}(t) = a_0 + a_1t + a_2t^2 + \dots + a_{k-1}t^{k-1}$. The coefficient matrix \mathbf{A}^{D_j} of this polynomial is obtained by:

$$\mathbf{A}^{D_j} = [a_0 \ a_1 \ a_2 \ \dots \ a_{k-1}]^T = (\mathbf{M}^{D_j})^{-1} \mathbf{B}^{D_j} \quad (12)$$

Here, \mathbf{M}^{D_j} and \mathbf{B}^{D_j} are defined as follows:

$$\mathbf{M}^{D_j} = \begin{bmatrix} 1 & (j-1)\tau & ((j-1)\tau)^2 & & ((j-1)\tau)^{k-1} \\ 0 & 1 & 2(j-1)\tau & \dots & (k-1)((j-1)\tau)^{k-2} \\ 0 & 0 & 2 & & (k-1)(k-2)((j-1)\tau)^{k-3} \\ & \vdots & & \ddots & \vdots \\ 0 & 0 & 0 & \dots & (k-1)! \end{bmatrix}$$

$$\mathbf{B}^{D_j} = \begin{bmatrix} u_n^{D_{j-1}}((j-1)\tau) \\ (u_n^{D_{j-1}})'((j-1)\tau) \\ (u_n^{D_{j-1}})''((j-1)\tau) \\ \vdots \\ (u_n^{D_{j-1}})^{(k-1)}((j-1)\tau) \end{bmatrix}$$

3. Numerical Validation

In this section, the proposed PIA(1,1) algorithm is applied to a benchmark delay differential equation to validate its accuracy and efficiency. The obtained semi-analytical results are compared with the exact solution and other numerical methods available in the literature to demonstrate the performance of the proposed approach.

Consider the second-order linear delay differential equation given by:

$$u''(t) + u(t) = u(t-1), \quad t \in [0,2] \quad (13)$$

subject to the history function and initial condition:

$$u(t) = t^2 + 3t + 2, \quad -1 \leq t \leq 0; \quad u'(0) = 0$$

According to the domain decomposition strategy described in Section 2, since the delay is $\tau = 1$ and the domain is $b = 2$, the problem is divided into $J = 2$ sub-intervals: $D_1 = [0,1]$ and $D_2 = [1,2]$. The global solution is constructed as:

$$u_n(t) = \begin{cases} u_n^{D_1}(t) & , \quad t \in D_1 \\ u_n^{D_2}(t) & , \quad t \in D_2 \end{cases}$$

Solution for the First Interval (D_1)

For $t \in [0,1]$, the delay term $u(t-1)$ falls into the history interval $[-1,0]$. Thus, the perturbation equation is constructed using the known history function $h(t)$:

$$(u^{D_1})'' + \varepsilon u^{D_1} - h(t-1) = 0$$

Substituting $h(t-1) = (t-1)^2 + 3(t-1) + 2$ and applying the PIA(1,1) expansion, the correction term ($u_c^{D_1}$) is derived. To determine the initial function $u_0^{D_1}(t)$, the boundary conditions at $t = 0$ are used. The coefficient matrix yields a simple starting function $u_0^{D_1}(t) = P_1^{D_1}(t) = 2$. Following the iteration process, the solution for the first interval is obtained as:

$$u_1^{D_1}(t) = \frac{t^4}{12} + \frac{t^3}{6} - t^2 + 2$$

$$u_2^{D_1}(t) = -\frac{t^6}{360} - \frac{t^5}{120} + \frac{t^4}{6} + \frac{t^3}{6} - \frac{t^2}{2} + 2$$

Solution for the Second Interval (D_2)

For $t \in [1,2]$, the delay term $u(t-1)$ depends on the solution $u^{D_1}(t)$ found in the previous step. The system equation becomes:

$$(u^{D_2})'' + \varepsilon u^{D_2} - u_n^{D_1}(t-1) = 0$$

Crucially, to ensure continuity at the node point $t = 1$, the initial function $u_0^{D_2}(t)$ is determined using the continuity conditions:

$$u_0^{D_2}(1) = u_n^{D_1}(1), \quad (u_0^{D_2})'(1) = (u_n^{D_1})'(1)$$

Using the matrix operation defined in Eq. (12), the coefficient matrix \mathbf{A}_{D_2} is calculated, yielding the initial function $u_0^{D_2}(t) = \frac{797}{360} - \frac{107}{120}t$. By employing this initial function in the PIA algorithm, the polynomial solution for the second interval is computed as follow:

$$u_1^{D_2}(t) = \frac{t^8}{20160} + \frac{t^7}{5040} - \frac{t^6}{180} - \frac{t^5}{120} + \frac{t^4}{6} + \frac{t^3}{6} - t^2 + 2$$

$$u_2^{D_2}(t) = -\frac{t^{10}}{1814400} - \frac{t^9}{362880} + \frac{t^8}{10080} + \frac{t^7}{5040} - \frac{t^6}{360} - \frac{t^5}{120} + \frac{t^4}{6} + \frac{t^3}{6} - t^2 + 2$$

Finally, combining the results from both steps, the global solution at the second iteration is expressed as:

$$u_2(t) = \begin{cases} -\frac{t^6}{360} - \frac{t^5}{120} + \frac{t^4}{6} + \frac{t^3}{6} - t^2 + 2 & , \quad t \in D_1 \\ -\frac{t^{10}}{1814400} - \frac{t^9}{362880} + \frac{t^8}{10080} + \frac{t^7}{5040} & , \quad t \in D_2 \\ -\frac{t^6}{360} - \frac{t^5}{120} + \frac{t^4}{6} + \frac{t^3}{6} - t^2 + 2 & \end{cases}$$

The results obtained by the PIA(1,1) algorithm are compared with the VMM (Martín & García, 2002) and the-DAM (Seong et al., 2013). Figure 1 presents the absolute errors at selected points.

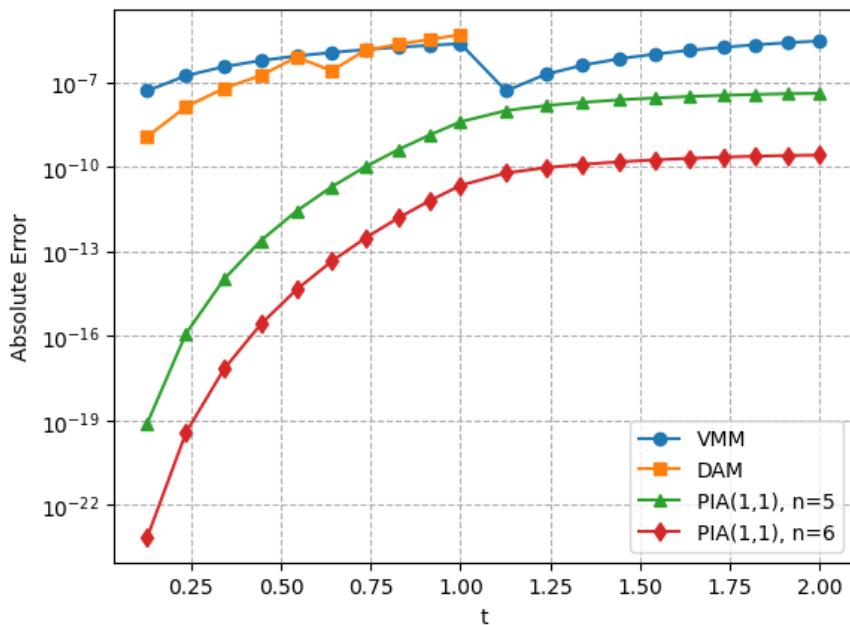


Figure 1. Comparison of absolute errors for PIA(1, 1) against VMM and DAM methods

As demonstrated in Figure 1, the PIA(1,1) algorithm achieves significantly lower error rates (10^{-16} to 10^{-24} range) compared to VMM and DAM (10^{-6} to 10^{-9} range). Furthermore, regarding computational cost defined by the “Totally Called Function” (FCN) count, PIA requires only 52 evaluations for $n = 5$ and 60 for $n = 6$, whereas DAM requires 61-73 evaluations. Here, FCN denotes the total number of function evaluations required by the algorithm and is used as a hardware-independent measure of computational cost. The maximum absolute error decreases exponentially with the number of iterations, achieving high precision in minimal CPU time (approx. 7.66s for $n = 6$).

The convergence speed is further analyzed in Table 1. It is observed that while the CPU time increases linearly with the number of iterations, the error decreases exponentially, achieving a maximum absolute error of 2.61×10^{-10} in just 7.66 seconds.

Table 1. Maximum absolute error and CPU time for different iterations

n	2	3	4	5	6
E_n	$1.25e - 02$	$3.10e - 04$	$4.44e - 06$	$4.05e - 08$	$2.61e - 10$
CPU time	2.69s	3.94s	5.26s	6.48s	7.66s

4. Applications in Computational Mechanics

In this section, the efficacy of the proposed PIA is demonstrated by applying it to complex engineering problems modeled by delay differential equations. Specifically, the method is employed to analyze the dynamic behavior of the DME and the DDME, which appear frequently in nonlinear vibration theory and control systems.

4.1. Dynamic analysis of the DME

The Mathieu equation is a quintessential model in the study of parametrically excited systems, describing phenomena such as the vibration of elliptical membranes and the stability of structures under periodic loading. A classic physical realization of this problem is a mathematical pendulum with a vertically moving support, as illustrated in Figure 2.

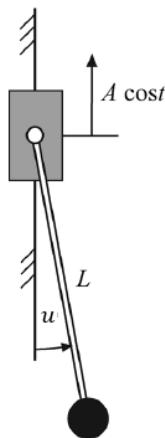


Figure 2. Mathematical pendulum with a vertically moving support (Kovacic et al., 2018)

When a linear control force is applied to stabilize such a system, the feedback mechanism inherently introduces a time delay. The equation of motion for this delayed system is given by:

$$u''(t) + (\delta + \varepsilon \cos t)u(t) = \zeta u(t - \tau) \quad (14)$$

where δ represents the square of the natural frequency, ε is the amplitude of parametric excitation, ζ is the delay coefficient, and τ is the time delay (taken as 2π).

4.1.1. Numerical implementation and comparison

The problem is solved over the domain $[0, 4\pi]$ using the PIA(1,1) algorithm with domain decomposition ($J = 2$). For parameters $\delta = 18$, $\varepsilon = 1$, and $\zeta = 0.5$, the semi-analytical solutions obtained by PIA are compared with the numerical results from the MATLAB dde23 solver.

As shown in Figure 3, the second and third iteration solutions of PIA exhibit excellent agreement with the numerical solution, confirming the high accuracy of the proposed method even for complex oscillatory behavior.

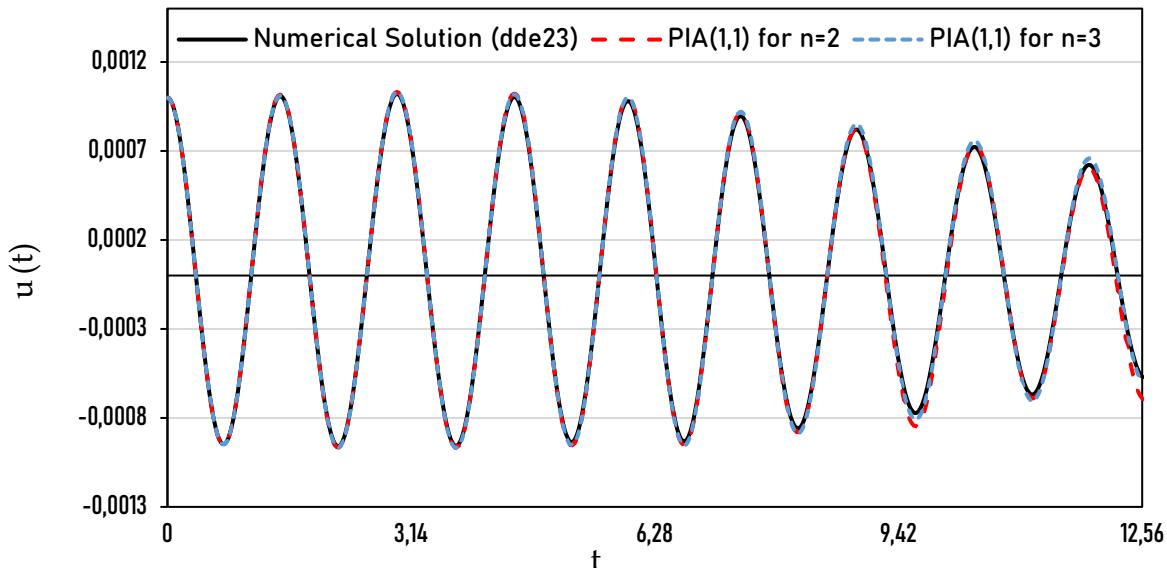


Figure 3. Comparison of the second and third iteration PIA(1,1) solutions with the MATLAB (dde23) numerical solution for the DME ($\delta = 18.0$, $\varepsilon = 1$, $\zeta = 0.5$, $\tau = 2\pi$)

4.1.2. Parametric analysis: Effect of delay and excitation

The influence of the delay coefficient (ζ) on the system's dynamics is analyzed in Figure 4. It is observed that increasing the delay coefficient (from $\zeta = 0$ to $\zeta = 1$) reduces the amplitude of oscillations. This phenomenon suggests that the delay term acts as a damping mechanism in the system. Mathematically, this can be approximated by the expansion $\zeta u(t - \tau) \approx \zeta u(t) - \zeta \tau u'(t)$, where the term $-\zeta \tau u'(t)$ introduces a velocity-dependent damping effect. It should be emphasized that this interpretation represents an approximate, first-order physical analogy intended to provide qualitative insight into the observed amplitude reduction, rather than an exact equivalence to classical viscous damping.

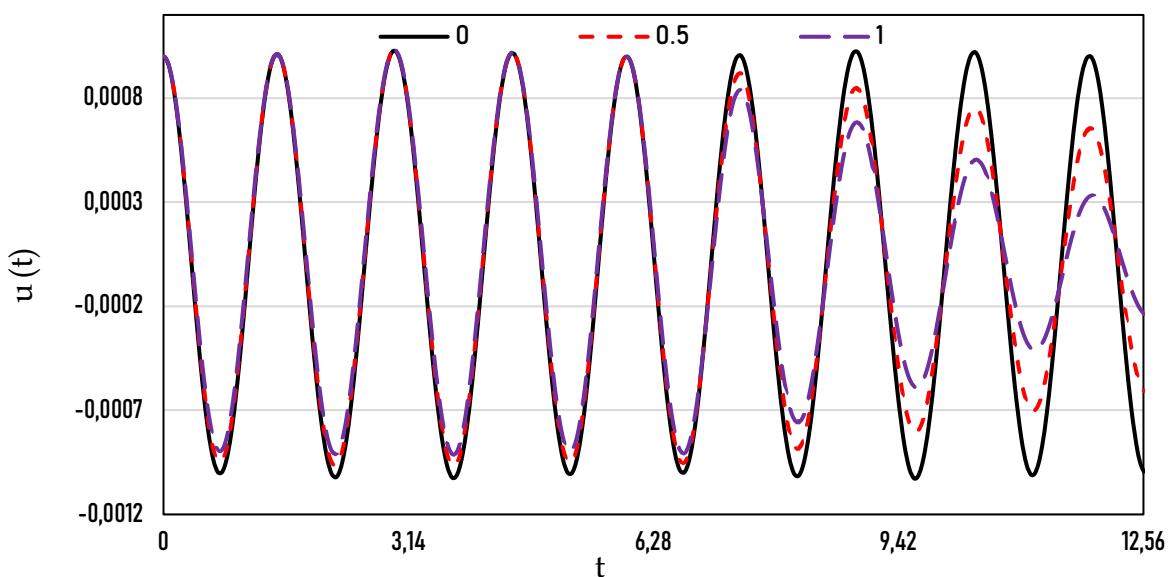


Figure 4. Fourth iteration PIA(1,1) solutions for different delay coefficients
 $\zeta = 0, 0.5, 1$ ($\delta = 18, \varepsilon = 1, \tau = 2\pi$)

The effect of the perturbation parameter ε , which represents the magnitude of the parametric excitation, is investigated in Figure 5 and Figure 6. As ε increases (e.g., from $\varepsilon = 1$ to $\varepsilon = 5$), the system becomes "stiffer," and the convergence rate of the algorithm decreases slightly, requiring higher iterations (e.g., $n = 6$) to match the numerical solution. Furthermore, as shown in Figure 6, increasing ε leads to a significant increase in the vibration amplitude.

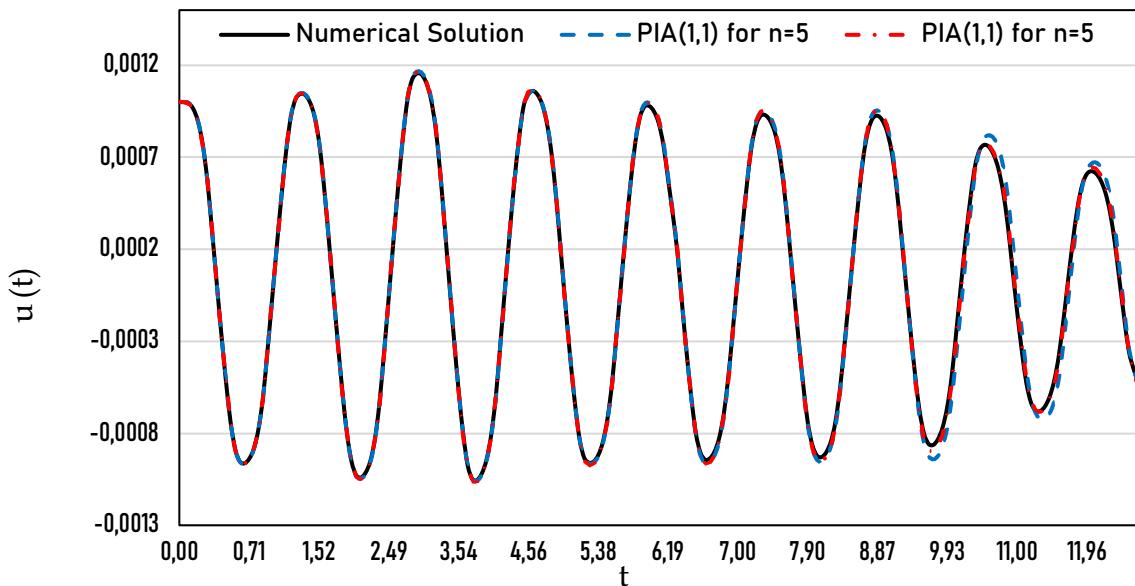


Figure 5. Comparison of PIA(1,1) solutions (5th and 6th iterations) with numerical results for high parametric excitation $\varepsilon = 5$

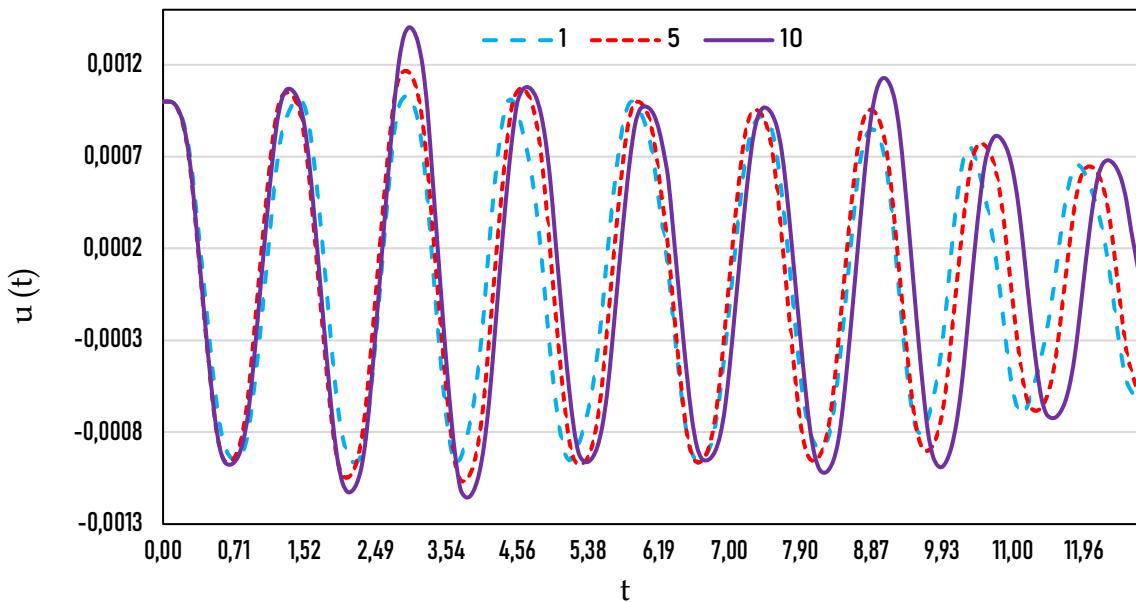


Figure 6. PIA(1,1) solutions for varying excitation amplitudes $\varepsilon = 1, 5, 10$
 $(\delta = 18, \zeta = 0.5, \tau = 2\pi)$

Finally, the effect of the natural frequency parameter δ is presented in Figure 7. Variations in δ directly alter the oscillation period of the system, demonstrating the method's capability to capture frequency shifts accurately.

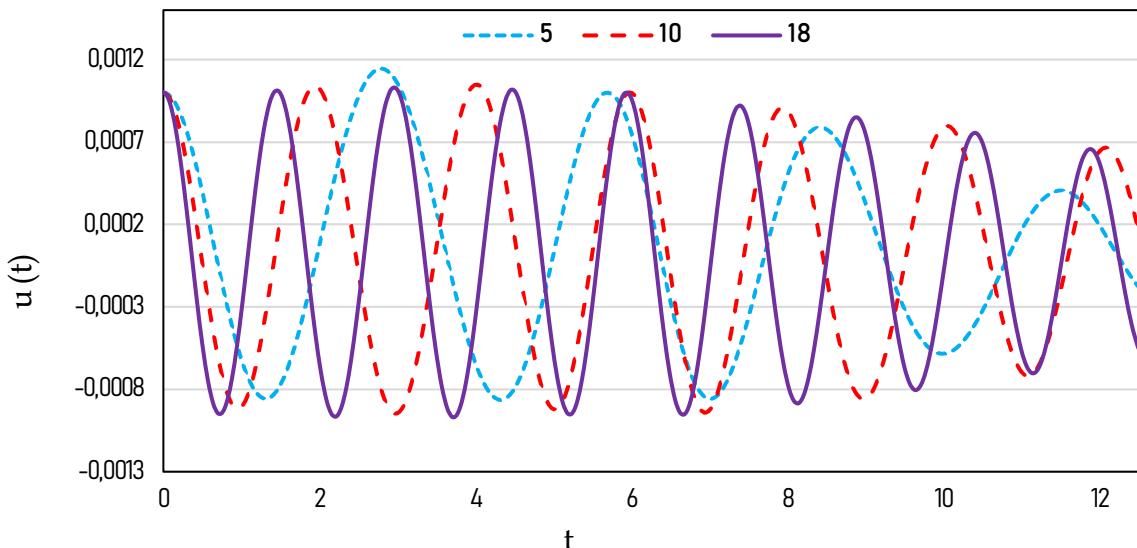


Figure 7. PIA(1,1) solutions for different δ values of 5, 10, and 18

4.2. Dynamic analysis of the DDME

To provide a more comprehensive analysis of engineering systems where both physical damping and time delays are present, we consider the DDME:

$$u''(t) + \kappa u'(t) + (\delta + \varepsilon \cos t)u(t) = \zeta u(t - \tau) \quad (15)$$

where κ is the physical damping coefficient.

The performance of the PIA(1,1) algorithm is compared against EMHPM (Olvera et al., 2015) and the standard numerical solver. Figure 8 presents the results for $\kappa = 0.2$, $\delta = 3.0$, $\varepsilon = 1$, and $\zeta = -1$. The comparison reveals that the PIA solution aligns more closely with the exact numerical solution than the EMHPM solution, highlighting the superior convergence properties of the proposed algorithm for damped delayed systems.

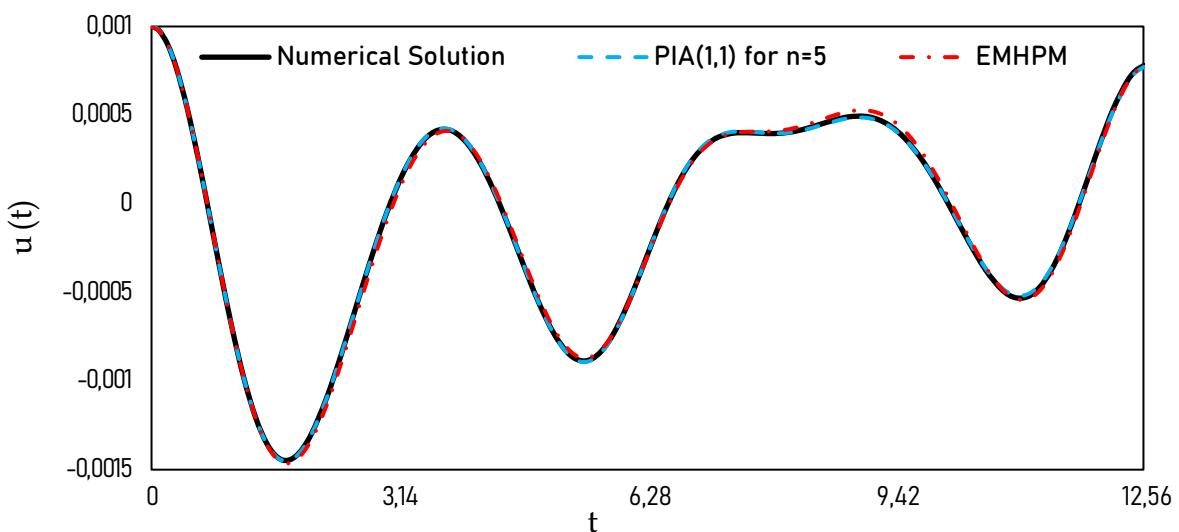


Figure 8. Comparison of PIA(1,1) (5th iteration), EMHPM (1st order), and numerical solutions for the DDME

The effect of the physical damping coefficient κ is illustrated in Figure 9. As expected, increasing κ rapidly decays the oscillation amplitude, validating the method's handling of the first-derivative term.

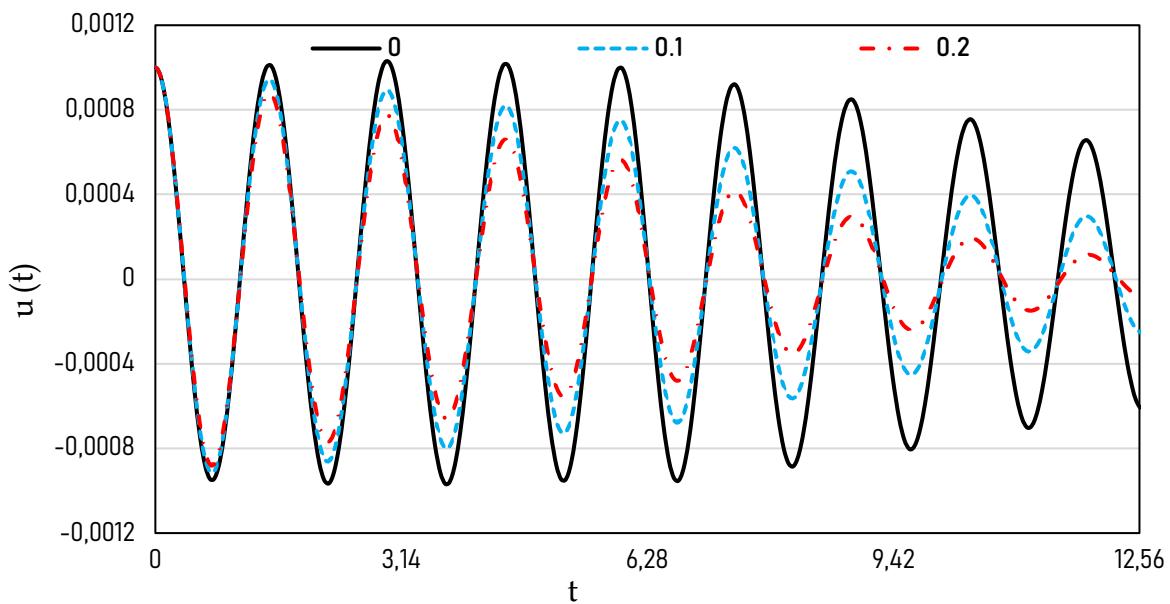


Figure 9. Fifth iteration PIA(1,1) solutions for different damping coefficients $\kappa = 0, 0.1, 0.2$

A critical analysis is performed in Figure 10, which examines the interplay between physical damping and the delay coefficient (ζ). While positive values of ζ (e.g., 0.5) reinforce the damping effect, a negative delay coefficient (e.g., $\zeta = -0.87$) counteracts the physical damping. This leads to a scenario where the system maintains a certain amplitude or approaches instability, despite the presence of physical damping. This capability to predict stability boundaries is crucial for the design of active control systems.

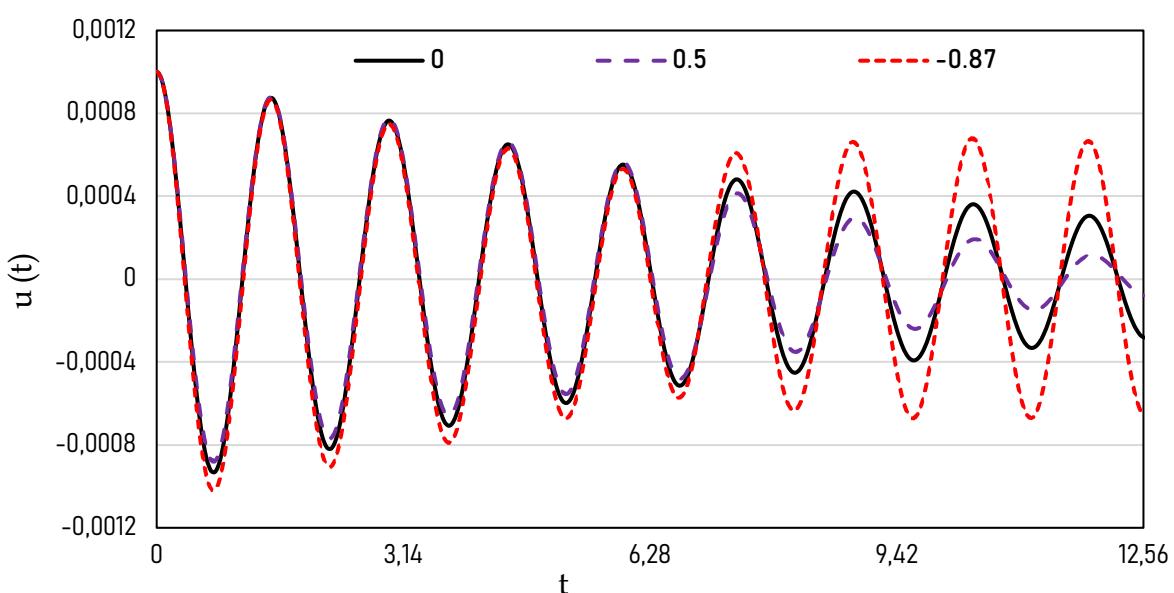


Figure 10. PIA(1,1) solutions for varying delay coefficients $\zeta = 0, 0.5, -0.87$ under physical damping $\kappa = 0.2$

5. Conclusion

In this study, the PIA(1,1) was successfully extended and applied to solve high-order delay differential equations. By integrating the method of steps with a robust continuity enforcement procedure based on matrix operations, the algorithm effectively manages the history functions and time delays inherent in such systems.

Numerical validation on a linear benchmark problem demonstrated that the proposed PIA(1,1) algorithm outperforms widely used techniques in the literature, such as the VMM and the DAM, in both solution accuracy and computational efficiency. The method has shown its suitability for computationally intensive engineering problems by achieving high-precision results with minimal CPU time.

Application of the method to the DME provided valuable physical insights into the stability of parametrically excited systems. Analysis of the results indicates that the delay term acts as an "artificial" damping mechanism; specifically, increasing the positive delay coefficient significantly reduces vibration amplitudes, thereby stabilizing the system. Conversely, investigations of the DDME revealed that negative delay coefficients act as an energy source counteracting physical damping, potentially driving the system towards instability. Furthermore, comparative analyses indicated that the PIA algorithm produces results closer to the exact numerical solution than the EMHPM, particularly for complex damped systems. In conclusion, this study confirms that the proposed PIA(1,1) algorithm is a powerful, reliable, and efficient mathematical tool for the dynamic analysis and design of linear and nonlinear engineering systems with time delays.

Despite its accuracy and efficiency for constant-delay problems, the proposed method is primarily suited to delay differential equations with fixed delays and moderate nonlinearity. For strongly nonlinear or high-dimensional systems, the size of the resulting algebraic systems and the computational cost may increase, potentially affecting efficiency. Moreover, extensions of the present framework to problems involving state-dependent delays are not considered in this study and remain an important direction for future research.

Authorship Contribution Statement

The author is solely responsible for the conceptualization, methodology, data collection, analysis, and manuscript preparation.

Conflict of Interest

The author declares no conflict of interest.

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