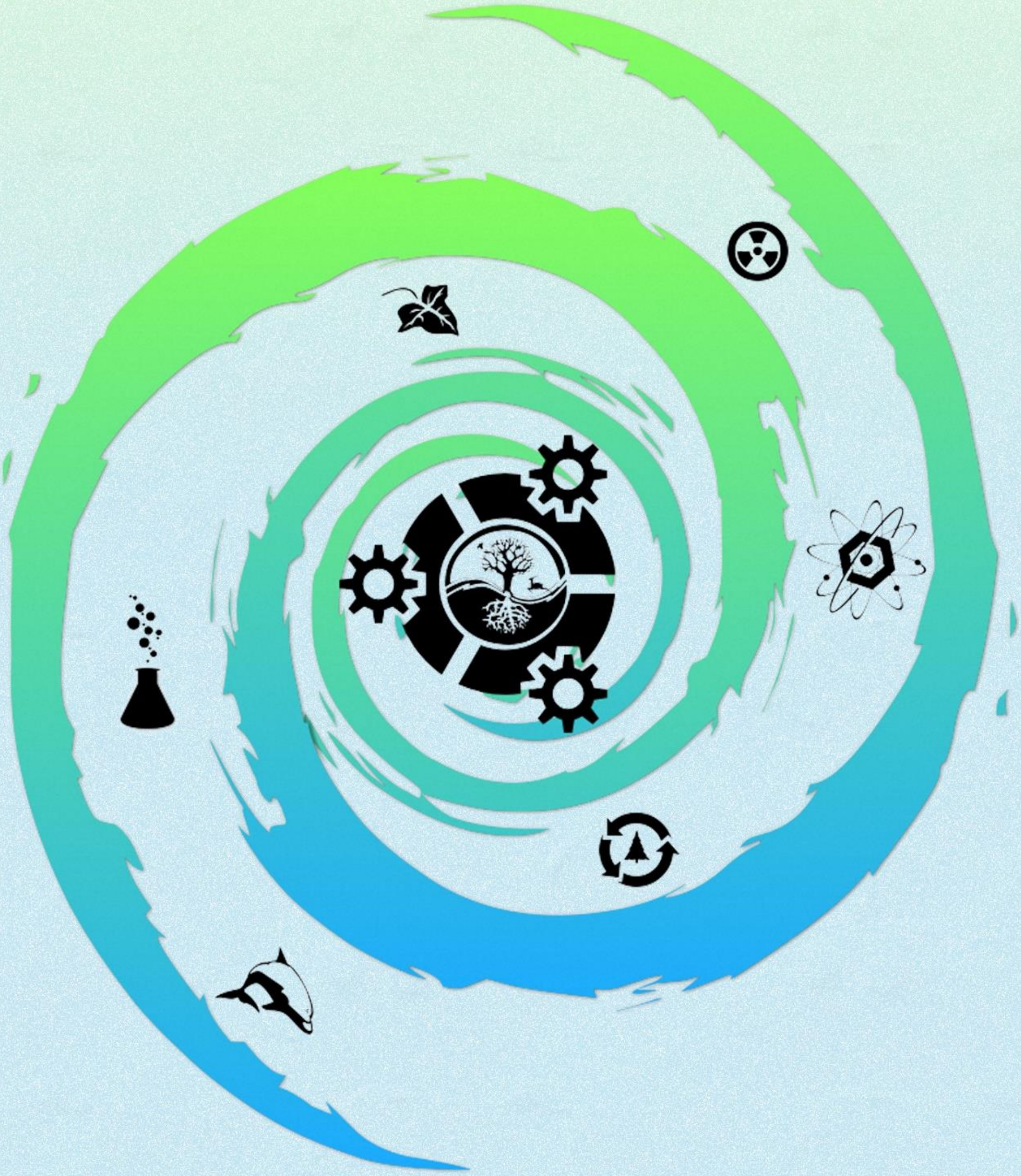
ACTA NATURA ET SCIENTIA

VOLUME: 7 ISSUE: 1 YEAR: 2026



e-ISSN: 2718-0638

www.actanatsci.com

PRENSIP

ACTA NATURA ET SCIENTIA

2026 • VOLUME 7 • ISSUE 1

ACTA NATURA ET SCIENTIA

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ACTA NATURA ET SCIENTIA 2026 • VOLUME 7 • ISSUE 1

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PUBLISHER

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Prensip Publishing and Consultancy Ind. Trade. Co. Ltd.
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37150, Merkez, Kastamonu, Türkiye
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DOI: 10.61326/actanatsci.v7i1.370
e-ISSN: 2718-0638

Review Article
info@actanatsci.com
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ACTA NATURA ET SCIENTIA PRENSIP

The Origin and Development of FMEAs in the North American Automotive Industry: A History Review

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Please cite this paper as follows:
Barsalou, M. (2026). The Origin and Development of FMEAs in the North American Automotive Industry: A History Review. *Acta Natura et Scientia*, *7*(1), 1-17. https://doi.org/10.61326/actanatsci.v7i1.370

ARTICLE INFO

Article History
Received: 29.05.2025
Revised: 04.10.2025
Accepted: 25.10.2025
Available online: 25.02.2026

Keywords:
Failure Modes and Effects Analysis
FMEA
Automotive industry
Risk analysis
Standards

ABSTRACT

The concept of Failure Modes and Effects Analysis has been around for almost three-quarters of a century, yet many authors give contradictory dates for the origin of the Failure Modes and Effects Analysis and different dates for the spread of Failure Modes and Effects Analysis in the automotive industry. This paper seeks to establish a timeline for the development of Failure Modes and Effects Analysis and Failure Modes and Effects Analysis usage in the North American automotive industry. This paper uses a historical review. Literature was identified and reviewed to document the development of the Failure Modes and Effects Analysis in the North American automotive industry using primary sources. The first standard for the Failure Modes and Effects Analysis was MIL-P-1629 in 1949. Failure Modes and Effects Analysis were used in the aerospace industry in the 1950s and the usage of Failure Modes and Effects Analysis at Ford Motor Company in 1972 and Toyota in 1975 has been confirmed. Automotive companies mandated use of the Failure Modes and Effects Analysis in the 1980s and automotive industry-specific standards were released in the 1990s and revised over the years. This paper contributes a timeline of key Failure Modes and Effects Analysis related events that is supported by documented evidence.

INTRODUCTION

An FMEA (Failure Modes and Effects Analysis) is widely used in industry (Xiao, 2011) and may be a PFMEA (Process Failure Modes and Effects Analysis) for a process or a DFMEA (Design Failure Modes and Effects Analysis) for a design (Anleitner, 2011). The FMEA is "a step-by-step approach for identifying all possible failures in a design, a manufacturing or assembly process, or a product or service" (Fonseca et al., 2015). The use of an FMEA is helpful for improving both the quality of products and delivery performance by preventing problems in both design and manufacturing (Ghadge et al., 2017).

The topic of FMEAs is well researched. For example, Zulfiqar et al. (2025) investigated the use of FMEAs in institutions of higher learning and Ali et al.

(2025) explored the use of FMEAs for the management of risks in cross-country pipelines when using Geographic Information Systems (GIS). Darmawan (2025) explains the use of FMEAs for Islamic banking product marketing Tangestani et al. (2025) describe the use of FMEAs as part of an indoor air pollutant risk assessment model for healthcare. Singh (2025) describes the application of FMEAs for improving work-life balance (WLB) in the healthcare sector in India and Kar & Rai (2025) describe the application of a fuzzy FMEA for a Six Sigma risk assessment in Quality 4.0. Risk prioritization in sustainable shipbuilding was described by Yılmaz & Köse (2025) using the integration of FMEAs and machine learning and Gandhare et al. (2025) describe the use of FMEAs for the development and validation of a medical equipment framework in Industry 4.0.

The origin of FMEAs is well-documented in the literature; however, much of the literature is contradictory. The first use of FMEAs has been attributed to the U. S. military in the 1940s (Manos & Vincent, 2012). Korenko et al. (2012) state that FMEAs started in 1949 with MIL-P-1629 and were then used in 1963 for NASA's Apollo program before entering the automotive industry through Ford in 1973.

According to Hatty & Owens (1994), the "FMEA was developed during the early 1960s in the U.S. aerospace industry" and the U.S. Department of the Army also credits NASA with developing the concept of FMEAs (2006). Sharma et al. (2007) attribute the origin of FMEAs to NASA in 1963. The development of FMEAs has also been attributed to the U.S. Navy's Bureau of Aeronautics in the early 1950s (Dhillon, 2003).

Ginn et al. (1998) attribute the origin of FMEAs to the aerospace industry in the mid-1960s and Plinta et al. (2021) state FMEAs were "born in the 1950s for the needs of the arms industry". The origin of FMEAs has also been attributed to the Grumman Aircraft Corporation in the 1950s (Bowles, 2002; Sharma & Sharma, 2010).

According to SAE (2009), FMEAs have been in use in the automotive industry since the 1960s, but the use of standard criteria for ranking and standard forms did not arrive till the 1990s. Carlson (2015) explains that Ford introduced the world to the automotive industry in the late 1970s due to the Ford Pinto situation and Anleitner (2011) states that the use of FMEAs became widespread at Ford in the mid-1970s due to the Pinto situation.

This paper seeks to establish a definitive timeline for the origin and development of FMEAs in the North American automotive industry.

An FMEA may be for a complete system, the design of a product, a manufacturing or assembly process (Carlson, 2015), or a service (Doshi & Desai, 2016). An FMEA uses multi-functional teams with members from various departments, such as quality, reliability, design, and manufacturing to identify and prevent failures before they occur (Maisano et al., 2020).

Failure modes, failure causes, and failure effects are identified in an FMEA. The failure mode is the way in which a process or product fails to fulfill the intended function of the product or process. Failure causes are what lead to the failure mode and failure effects are the consequence of the failure mode (Pillay & Wang, 2003). The failure effects may be identified for a component, subassembly, or complete system. Prevention actions are identified to prevent the failure cause and detection actions are identified to detect the failure mode or failure cause (Correia dos Santos et al., 2012).

The severity is rated with a value from one to ten based on how bad the consequences of the failure are. The occurrence of the failure cause is rated on a scale of one to ten based on how well the failure cause can be prevented. Detection is also rated on a scale of one to ten based on how well the failure cause or failure mode can be detected. The severity, occurrence, and detection ratings are determined using tables (Maisano et al., 2020). Table 1 depicts a severity table, Table 2 depicts an occurrence table, and Table 3 depicts a detection table. Different standards and different organizations may use different tables.

Table 1. Severity table reproduced from Department of the Army (2006). *TM 5-698-4, Failure Modes, Effects and Criticality Analyses (FMECA) for Command, Control, Communications, Computer, Intelligence, Surveillance, and Reconnaissance (C4ISR) Facilities*.

Ranking	Effect	Comment
1	None	No reason to expect failure to have any effect on Safety, Health, Environment or Mission.
2	Very Low	Minor disruption to facility function. Repair to failure can be accomplished during trouble call.
3	Low	Minor disruption to facility function. Repair to failure may be longer than trouble call but does not delay Mission.
4	Low to Moderate	Moderate disruption to facility function. Some portion of Mission may need to be reworked or process delayed.
5	Moderate	Moderate disruption to facility function. 100% of Mission may need to be reworked or process delayed.
6	Moderate to High	Moderate disruption to facility function. Some portion of Mission is lost. Moderate delay in restoring function.
7	High	High disruption to facility function. Some portion of Mission is lost. Significant delay in restoring function.
8	Very High	High disruption to facility function. All of Mission is lost. Significant delay in restoring function.
9	Hazzard	Potential Safety, Health or Environmental issue. Failure will occur with warning.
10	Hazzard	Potential Safety, Health or Environmental issue. Failure will occur without warning.

Table 2. Occurrence table reproduced from Department of the Army. (2006). *TM 5-698-4, Failure Modes, Effects and Criticality Analyses (FMECA) for Command, Control, Communications, Computer, Intelligence, Surveillance, and Reconnaissance (C4ISR) Facilities*.

Ranking	Failure Rate	Comment
1	1/10,000	Remote probability of occurrence; unreasonable to expect failure to occur.
2	1/5,000	Very low failure rate. Similar to past design that has, had low failure rates for given volume/loads.
3	1/2,000	Low failure rate based on similar design for given volume/loads.
4	1/1,000	Occasional failure rate. Similar to past design that has had similar failure rates for given volume/loads.
5	1/500	Moderate failure rate. Similar to past design having moderate failure rates for given volume/loads.
6	1/200	Moderate to high failure rate. Similar to past design having moderate failure rates for given volume/loads.
7	1/100	High failure rate. Similar to past design having frequent failures that caused problems.
8	1/50	High failure rate. Similar to past design having frequent failures that caused problems.
9	1/20	Very high failure rate. Almost certain to cause problems.
10	1/10+	Very high failure rate. Almost certain to cause problems.

Table 3. Detection table reproduced from Department of the Army. (2006). *TM 5-698-4, Failure Modes, Effects and Criticality Analyses (FMECA) for Command, Control, Communications, Computer, Intelligence, Surveillance, and Reconnaissance (C4ISR) Facilities*.

Ranking	Detection	Comment
1	Almost Certain	Current control(s) almost certain to detect failure mode. Reliable controls are known with similar processes.
2	Very High	Very high likelihood current control(s) will detect failure mode.
3	High	High likelihood current control(s) will detect failure mode.
4	Moderately High	High Moderately high likelihood current control(s) will detect failure mode.
5	Moderately High	Moderate likelihood current control(s) will detect failure mode.
6	Low	Low likelihood current control(s) will detect failure mode.
7	Very Low	Very low likelihood current control(s) will detect failure mode.
8	Remote	Remote likelihood current control(s) will detect failure mode.
9	Very Remote	Very remote likelihood current control(s) will detect failure mode.
10	Almost Impossible	No known control(s) available to detect failure mode.

The severity, occurrence, and detection values are multiplied to derive an RPN (Risk Priority Number) that is used for prioritizing improvements (Doshi & Desai, 2016). A known weakness of FMEAs prioritized using RPN is that severity, occurrence, and detection all have equal weight (Pillay & Wang, 2003; Sharma et al., 2007; Maisano et al., 2020). A solution to this was introduced by AIAG/VDA (2019) with the use of Action Priority (AP) in place of RPN. In place of multiplying severity times occurrence times detection to calculate an RPN for prioritization, AP is derived using tables for severity, occurrence, and detection, with the greatest emphasis on severity, followed by occurrences and then detection with the least emphasis (AIAG/VDA, 2019).

MATERIAL AND METHODS

This paper is a historical review, which is used to follow the history of a topic (Collins & Fauser, 2005). The objective was not to perform a systematic review that seeks to capture all available research. The approach used is a semi-systematic review, which is a method used for gaining an overview or historical events and establishing a timeline (Snyder, 2019). Databases used to identify papers were from Blackwell, Routledge, Elsevier, Taylor and Francis, and Science Direct, as well as American Society for Quality (ASQ's) database of ASQ journals. The search terms included "Failure Modes and Effects Analysis," "Failure Modes Effects and Criticality Analysis," "FMEA," and "FMECA." An internet search was also performed to locate copies of original documents that were identified in the papers that were reviewed. Target documents included copies of original guides and standards, contemporaneous accounts, and firsthand accounts that were published after the event in question. The search objective was to identify the dates of key developments in the history of FMEAs to establish a timeline of developments in FMEAs in the North American automotive industry.

The Emerald Insight database was also used to identify changes over time in the number of publications related to both FMEAs and FMECAs (Failure Mode Effects and Criticality Analysis). The search terms "Failure Modes and Effects Analysis" and "Failure Modes Effects and Criticality Analysis" were both used to identify the number of publications in five-year intervals, starting with 1949, which was the year in which FMECAs were introduced. Using five-year intervals starting in 1949 and ending in the year before the study was performed ensured all possible full years were covered.

The concept of an FMEA was introduced in 1949 by *Procedures for Performing a Failure Mode, Effects and Criticality Analysis Mil-P-1629* (Department of Defense, 1949). An FMECA is much like an FMEA, but uses a criticality calculation (Carlson, 2015). The standard was later canceled in 1963 (Department of Defense, 1963).

An FMEA standard was published in 1966 for the Apollo program and the standard described an FMEA together with a criticality analysis, as well as use of a block diagram. The criticality shown in Eq. 1 was calculated as:

$$C_r = \sum_{n=1}^{j} (\beta \alpha K_E K_A \lambda_G \; t \; \times 10^6)_n \,, \mathrm{n} = 1, 2, 3, \ldots, \mathrm{j} \qquad (1)$$

where C_r is criticality, j is the number of critical failure modes, β is the conditional probability that the failure effects occur if a failure mode has occurred, α is the fraction of failures due to the failure mode, K_E is an environmental factor that adjusts λ_G for the difference between environmental stresses where λ_G was measured and λ_G will be used, K_A is an operational factor that adjusts λ_G for the difference between operational stresses where λ_G was measured and λ_G will be used, λ_G is a failure rate in hours or cycles, t is operating time in hours or cycles, and n is an index summation of critical failure modes of a component (NASA, 1966).

An FMEA standard, *ARP926 Fault/Failure Analysis Procedure*, was published by SAE in 1967 and this was followed by *MIL-STD-1629 (ships) Procedures for Performing a Failure Mode, Effects and Criticality Analysis*, in 1974 (Department of Defense, 1974), which was superseded by MIL-STD-1629a in 1980. MIL-STD-1629A used two approaches for determining criticality. One was a qualitative approach with five levels for when data was unavailable. The levels were:

- Level A - Frequent: Failure probability > 0.20
- Level B – Reasonably probable: Failure probability 0.10 - 0.20
- Level C - Occasional: Failure probability > 0.01 to < 0.10
- Level D - Remote: Failure probability > 0.001 to < 0.01
- Level E - Extremely unlikely – Failure probability < 0.001 (Department of Defense, 1980)

The quantitative approach shown in Eq. 2 used calculated criticality as:

$$C_m = \beta \alpha \lambda_p \; t, \qquad (2)$$

where C_m is the criticality for the failure mode, β is the conditional probability of mission loss, α is the failure mode ratio, λ_p is the part failure rate, and t is operating duration in hours or cycles (Department of Defense, 1980).

Ford Motor Company has been credited with bringing FMEAs into the automotive industry in 1977 (VDA, 2012). However, Termaat & Freeman (1972) mention the use of an FMEA for the development of a crash sensor at Ford Motor Company in 1972 and Durstine (1973) reported on the use of an FMEA for the development of a truck steering system at Ford Motor Company in 1973. The use of a scale of one to ten for rating severity, occurrence, and detection to derive an RPN at Ford Motor Company was described in 1975 for the third generation of the Econoline van by Walsh (1975), who called FMEAs a "key reliability tool" used for design, processes, and services.

The use of FMEAs for the development of catalectic converters at Toyota Motor Company was documented in 1975. The form sheet included function and failure mode as well as columns for the failure effect at the subsystem and at the engine. In place of an RPN, the FMEA used criticality, which was derived by multiplying the intensity of damage with a correction factor for how quickly a repair is expected (Matsumoto et al., 1975)

According to Smith (2005), who retired from Ford Motor Company after 27 years in quality, there was a "Major push on failure modes and effects analysis (FMEA)" at Ford Motor Company between 1977 and 1980. Ford Motor Company released an FMEA training video in 1986 and Jaguar Cars Limited released an instruction guide for FMEAs in 1988 (Aldridge et al., 1991). Ford Motor Company then released *Potential Failure Mode and Effects Analysis in Design (Design FMEA) and for Manufacturing and Assembly Processes (Process FMEA) Instruction Manual* in 1988 (SAE, 2001).

Automotive companies had their own standards for suppliers to fulfill in the early 1990s, which resulted in suppliers needing to conform to multiple standards when delivering to different customers. As a result, QS-9000 was developed for the automotive industry (Fong & Antony, 2001) based on collaboration between Chrysler Corporation, Ford Motor Company, and General Motors Corporation with technical experts (Manos & Vincent, 2012).

The standard *QS-9000* contained automotive industry-specific additions to ISO 9000 and included a requirement for FMEAs (Stamatis, 1996). The standard was introduced in 1994 and mandated use of DFMEAs and PFMEAs and referred readers to *Potential Failure Mode and Effects Analysis Reference Manual* for guidance (Chrysler Corporation et al., 1998).

In addition to *QS-9000*, there were additional automotive industry quality standards worldwide, from organizations such as VDA, EAQF, and AVSQ; therefore, these were harmonized with ISO/TS 16949 (Fong & Antony, 2001). The current version is *IATF 16949:2016* and the use of FMEAs is still mandated (IATF, 2016).

The *Potential Failure Mode and Effects Analysis Reference Manual* was initially released in 1993 and revised multiple times with a fourth and last edition released in 2008 (Chrysler LLC et al., 2008) and then superseded by *AIAG/VDA FMEA Handbook* in 2019 (AIAG/VDA, 2019). A VDA standard for FMEAs in the German automotive industry was released in 1996 and the approach was different than the approach used in the North American automotive industry (Fritzsche, 2011). The release of *AIAG/VDA FMEA Handbook* resulted in a harmonization of VDA and AIAG approaches to FMEAs (Plinta et al., 2021).

A 1988 fact-finding mission to Japan found a widespread use of FMEAs (Atkinson, 1989) and according to Henshall (1989), Ford Motor Company in Europe was using FMEAs to identify special characteristics that would be controlled through Statistical Process Control (SPC) in 1989. The use of FMEAs with RPNs at Garrett Automotive Group in 1991 was described by Aldridge et al. (1991), who reported FMEAs were used due to Original Equipment Manufacturers (OEMs) requiring FMEAs.

Swift & Flynn (1989) recommend the use of a PFMEA with a standardized form sheet. The authors presented an example for a form sheet, which included columns for function, failure mode, mechanism and cause of failure, effect of failure, and one column for current controls, as well as columns for recommend and taken actions and a re-evaluation. However, in place of RPN, the authors used risk priority measure, which was derived by multiplying S for seriousness, D for likelihood of a customer receiving a defect, and P for the probability of an occurrence. The authors also provided an evaluation table with scores ranging from one to five for S, D, and P.

A study by Dale & Shaw (1990) on 78 automotive industry organizations in the United Kingdom found that most organizations only implemented the use of FMEAs due to customer requirements; however, some organizations were seeking to apply FMEAs for continuous improvement. The study also found the need for software for FMEAs. The use of software for FMEAs was also described in 1991 (Webber, 1990).

By 1992, there was also an FMEA guide for the semiconductor industry with RPN, severity, occurrence, and detection evaluation tables and a form sheet comparable (Villacourt, 1992) to later form sheets (Chrysler LLC et al., 2008; SAE, 2009). A 1994 FMEA example displays a form sheet (Hatty & Owens, 1994) that aligns with the later versions of the form sheet (Chrysler LLC et al., 2008; SAE, 2009).

In 1998 MIL-STD-1629A was canceled with a recommendation to refer to national and international documents for FMECA guidance (Department of Defense, 1998) due to a memorandum from the Secretary of Defense instructing the Department of Defense to increase the use of commercial products and commercial practices (SAE, 2001).

The use of a boundary diagram for DFMEAs and process flow chart for PFMEAs was added to the *SAE J1739* in the 2009 version due to increased use of those tools for FMEAs in industry (SAE, 2009). The boundary diagram for DFMEAs and flow chart were both recommended in the 2001 third edition of *Potential Failure Modes and Effects Analysis (FMEA): Reference Manual* (DaimlerChrysler Corporation et al., 2001). The use of a boundary diagram was described as far back as 1992 in a semiconductor industry standard (Villacourt, 1992). The p-diagram (parameter diagram) was included in the fourth edition of *Potential Failure Modes and Effects Analysis (FMEA): Reference Manual (*Chrysler LLC et al., 2008), but was not mentioned in 2002 edition of *SAE J 1739* (SAE, 2002).

The approach to FMEAs used in *Potential Failure Modes and Effects Analysis (FMEA): Reference Manual* and the German *VDA Volume 4* differed significantly, resulting in two sets of FMEA standards. This was remedied with a harmonized approach in *AIAG/VDA FMEA Handbook* released in 2019 (Plinta et al., 2021). The *Potential Failure Modes and Effects Analysis (FMEA): Reference Manual* used flow charts for PFMEAs and boundary diagrams and p-diagrams for DFMEAs with a simple form sheet (Chrysler LLC et al., 2008). The VDA approach did not use boundary diagrams and p-diagrams for DFMEAs and flow charts for PFMEAs. Instead, a structure analysis was performed to break products and processes down from the complete system to the individual elements. A function analysis was then performed to assign functions to every level of the product or process. A failure analysis was then performed to assign failures to functions (VDA, 2012). The VDA approach was more complicated and required the use of software.

AIAG/VDA Failure Modes and Effects Handbook harmonized AIAG and VDA approaches with a new approach that could be done either using a spreadsheet, or in FMEA software. The VDA structure, function, and failure analysis are required; however, they can either be performed in FMEA software or completed in the form sheet. The use of a boundary diagram and p-diagram for DFMEAs is only needed if a structure tree and function tree created in FMEA software is not used. For PFMEAs, a structure tree in software can be used, or a flow chart for PFMEAs that are not created in FMEA software. In place of an RPN, an AP is used for prioritization (AIAG/VDA, 2019).

The standard SAE J1739 was updated in 2021 with the release of *Surface Vehicle Standard J1739: (R) Potential Failure Mode and Effects Analysis (FMEA) Including Design FMEA, Supplemental FMEA-MSR, and Process FMEA*. The update included boundary diagrams, p-diagrams, and flow charts. The update also included *AIAG/VDA FMEA Handbook* severity, occurrence, and detection tables as well as AP tables for determining AP, but with the option to choose between use of AP or RPN (SAE, 2021).

RESULTS

The first FMEA standard was *MIL-P-1629* released by the U.S. Department of Defense in 1949. A standard was released by NASA in 1966, which was followed by an SAE standard for aerospace, *SAE ARP 926* in 1967. The U. S. Department of Defense *released MIL-STD-1629 (ships)* in 1974, which was canceled and later replaced by *MIL-STD-1629A*.

The use of FMEAs in the automotive industry in the early 1970s was confirmed with a publication describing the use of FMEAs at Ford Motor Company in 1972 (Termaat & Freeman, 1972) and Toyota Motor Company in 1975 (Matsumoto et al., 1975). FMEAs were used in the automotive industry in the 1980s (Aldridge et al., 1991) and Fong & Antony (2001) explain that suppliers were confronted with multiple standards, resulting in QS-9000 in 1994. The *QS-9000* standard transitioned into *ISO/TS 16949* and then *IATF 16949*. The standards required FMEAs, but did not specify how to do an FMEA. Guidance was provided in *FMEA Manual* and then *SAE J1739*. Both standards underwent revisions, with the AIAG's fourth edition being replaced by *AIAG/VDA FMEA Handbook*, which harmonized AIAG and VDA approaches to FMEAs. *SAE J1739* was also updated in 2021. A timeline of major FMEA events and standards is shown in Figure 1.

DISCUSSION

The 1966 standard used by NASA has some overlap with a modern FMEA, but also significantly differs, with NASA using the criticality approach. The columns in the NASA standard (NASA, 1966) bear resemblance to the columns in the 1980 *MIL-STD-1629*, which also used criticality (Department of Defense, 1980). The FMEA described at Ford in 1973 bore a strong resemblance to modern FMEAs, but with separate columns for the effect of failure on a part and system and failure effect on the vehicle as well as only one column for fixes, if available, but no prevention or detection action control column

(Durstine, 1973). The columns in FMEAs used by NASA in 1966, Ford in 1973, and *MIL-STD-1629A* are shown in Table 4.

The *Potential Failure Modes and Effects Analysis (FMEA): Reference Manual* (Third Edition) provided one form sheet for DFMEAs and one form sheet for PFMEA and suggested use of a block diagram for DFMEs and a process flow chart for PFMEAs (Chrysler Corporation et al., 1998). The *Potential Failure Modes and Effects Analysis (FMEA): Reference Manual* (Fourth Edition) offered six possible DFMEA forms labeled A through E and eight different PFMEA forms labeled A through H. For DFMEAs, the main differences are in how item, function, and requirements are listed, with one version listing all three in one column and other versions listing different combinations of two out of the three in one column and the third in a different column. All but one version has current design controls for prevention and detection in separate columns; however, there is also a version that lists current design controls above a column for cause and a column for failure mode. The PFMEA form sheets have various combinations of process step, function, and requirements. There are also two form sheets with ID, product, and process listed in columns underneath process. Comparable to the DFMEA form sheet, there is also a column for cause and a column for failure mode listed underneath current detection process controls. The use of either a block diagram or a p-diagram was suggested for DFMEAs and PFMEAs were recommended to use a process flow diagram. The DFMEA failure effects were required to be described from the view of the end user and PFMEA failure effects were to consider customers, including internal and external customers (Chrysler LLC et al., 2008).

Table 4. Content of form sheets in early FMEAs

Procedure for Failure Mode. Effects, and Criticality Analysis (FMECA) **(NASA, 1966)**	**FMEA at Ford (Durstine, 1973)**	***Military Standard: Procedures for Performing a Failure Mode, Effects and Criticality Analysis MIL-STD-1629A*** **(Department of Defense, 1980)**
Name	Failure Mode	Identification Number
Ident. Number	Failure Cause(s)	Item/ Functional Identification (Nomenclature)
Reliability Logic Diagram Number	Failure Effect on Part / System	Function
Function	Failure Effect on Vehicle	Failure Modes and Causes
Failure Mode and Cause	Probability of Failure Mode Occurrence	Mission Phase/ Operational Mode
Mission Phase	Probability of Severe Consequences	Failure Effects Local Effects
Failure Effect on Component / Functional Assembly	Probability of Failure Detection	Failure Effects Next Higher level
Failure Effect on Subsystem	Risk Priority Number	Failure Effects End User
Failure Effect on System	Present Fixes, if Any	Failure Detection method
Failure Detection Method	Recommended Corrective Action and Remarks	Compensating Provisions
Corrective Action Time Available/ Time required		Severity Class
Useful Life		Remarks

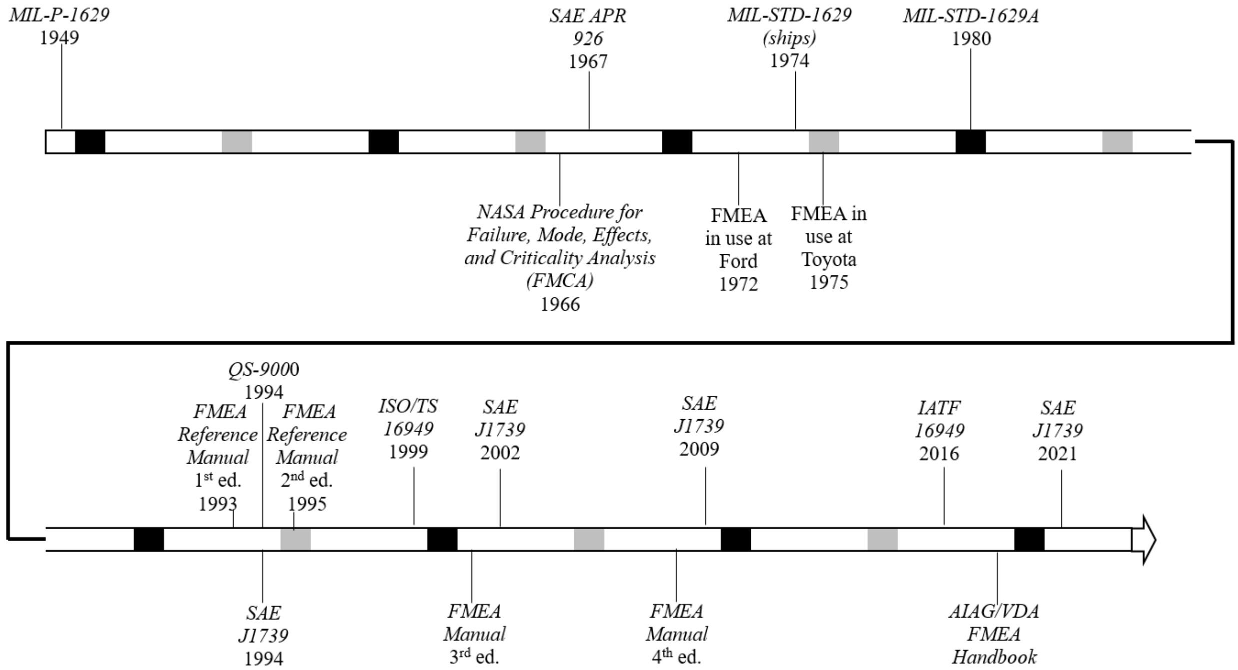

Figure 1. Timeline of major FMEA events and standards (by author)

The 2002 version of *SAE J1739* stated that a PFMEA should start with a flow chart and a DFMEA should begin with a block diagram, but no mention is made of a p-diagram. There were two possible form sheets for both DFMEAs and PFMEAs; one had current controls for prevention and detection combined in one column, and the other had current controls for prevention and detection in two separate columns (SAE, 2002). The 2009 revision of *SAE J1739* suggests a block diagram or boundary diagram for DFMEAs and a process flow diagram for PFMEAs. There was only one form sheet for DFMEAs and one form sheet for PFMEAs and both had separate columns for prevention and detection controls. The failure effects for the DFMEA considered the next assembly, the finished product, and the customer and the PFMEA failure effects pertain to the next operation, customer operations, and the end user (SAE, 2009).

The form sheets for DFMEAs and PFMEAs used in the 2008 edition of *Potential Failure Modes and Effects Analysis (FMEA): Reference Manual* and the 2009 edition of *SAE J1739* are shown in Table 5. The two standards were aligned (Chrysler LLC et al., 2008) and the main difference is in terminology with SAE J1739 including the term requirement together with item and function for DFMEAs and using abbreviations for severity, occurrence, and detection.

The *AIAG/VDA FMEA Handbook* offers a standard DFMEA form sheet, an alternative DFMEA form sheet, and a software view DFMEA form sheet, as well as a standard DFMEA MSR (Monitoring and System Response) form sheet and a software view DFMEA MSR form sheet. There is a standard PFMEA form sheet as well as four alternate form sheets and a PFMEA software view form sheet. The *AIAG/VDA FMEA Handbook* requires either a structure tree in software or a boundary diagram and the structure listed in the form sheet, as well as either a function tree in software, or a p-diagram with functions listed in the form sheet. The failure effects for the DFMEA can be for either the next higher level in the system, the end user, or both the next level in the system and the end user. The failure effects for a PFMEA are for the plant, the plant the part is shipped to, and the end user. The RPN was replaced with AP for prioritization (AIAG/VDA, 2019). The latest version of *SAE J1739* only has one DFMEA and PFMEA form sheet as well as a DFMEA MSR. Block diagrams and p-diagrams are recommended for DFMEAs and process flow diagrams for PFMEAs. The failure effects are the same as in the previous edition. Either RPN or AP can be used for prioritization. (SAE, 2021).

Table 5. Content of later FMEA form sheets

DFMEA Form A - Potential Failure Modes and Effects Analysis (FMEA): Reference Manual Fourth Edition (Chrysler LLC et al., 2008)	**PFMEA Form A - Potential Failure Modes and Effects Analysis (FMEA): Reference Manual Fourth Edition (Chrysler LLC et al., 2008)**	**DFMEA- SAE J1739 (SAE, 2009)**	**PFMEA- SAE J1739 (SAE, 2009)**
Function / Item	Process Step / Function / Requirements	Item / Function / Requirement	Process Step / Function / Requirement
Potential Failure Mode	Potential Failure Mode	Potential Failure Mode	Potential Failure Mode
Potential effect(s) of Failure	Potential effect(s) of Failure	Potential Effects(s) of Failure	Potential Effects(s) of Failure
Severity	Severity	SEV	SEV
Classification	Classification	Classification	Classification
Potential Cause(s) of Failures	Potential Cause(s) of Failures	Potential Cause(s) of Failure	Potential Cause(s) of Failure
Occurrence	Occurrence	OCC	OCC
Current Design Controls Prevention	Current Process Controls Prevention	Current Design Controls Prevention	Current Process Controls Prevention
Current Design Controls Detection	Current Process Controls Detection	Current Design Controls Prevention	Current Process Controls Prevention
Detection	Detection	DET	DET
RPN	RPN	RPN	RPN
Recommended Action	Recommended Action	Recommended Action	Recommended Action
Responsibility Target and Completion Date	Responsibility Target and Completion Date	Responsibility & Target Completion Date	Responsibility & Target Completion Date
Action Results Action Taken and Effective date	Action Results Action Taken and Effective date	Action Results Actions Taken & Effective Date	Action Results Actions Taken & Effective Date
Action results Severity	Action Results Severity	Action Results SEV	Action Results SEV
Action Results Occurrence	Action Results Occurrence	Action Results OCC	Action Results OCC
Action Results Detection	Action Results Detection	Action Results DET	Action Results DET
Action Results RPN	Action Results RPN	Action Results RPN	Action Results RPN

Table 6 depicts the form sheets for *AIAG/VDA FMEA Handbook* and SAE J1739. The latest editions of the two standards have diverged significantly in the form sheet with *AIAG/VDA FMEA Handbook* having many more columns to accommodate changes required to harmonize with the VDA approach.

Table 6. Content of the latest FMEA form sheets

Standard DFMEA - ***AIAG/VDA FMEA Handbook*** **(AIAG/VDA, 2019)**	**Standard PFMEA -** ***AIAG/VDA FMEA Handbook*** **(AIAG/VDA, 2019)**	**DFMEA -** ***SAE J1739*** **(SAE, 2021)**	**PFMEA-** ***SAE J1739*** **(SAE, 2021)**
Issue #	Issue #	DFMEA Technical Risk Analysis Item	Technical Risk Analysis Item (Op-Seq)
Continuous Improvement History / Change Authorization (As Appropriate)	Continuous Improvement History / Change Authorization (As Appropriate)	DFMEA Technical Risk Analysis Function(s)	Technical Risk Analysis Process Function
Structure Analysis (Step 2) 1. Next Higher Level	Structure Analysis (Step 2) 1. Process Item System, Subsystem, Part Element or Name of Process	DFMEA Technical Risk Analysis Potential Failure Mode(s)	Technical Risk Analysis Requirements
Structure Analysis (Step 2) 2. Focus Element	Structure Analysis (Step 2) 2. Process Step Station No. and Name of Focus Element	DFMEA Technical Risk Analysis Potential Effect(s) of Failure	Technical Risk Analysis Potential Failure Mode
Structure Analysis (Step 2) 3. Next Lower level or Characteristic Type	Structure Analysis (Step 2) 3. Process Work Element 4M Type	DFMEA Technical Risk Analysis Potential Cause(s) of Failure	Technical Risk Analysis Potential Effects of Failure
Function Analysis (Step 3) 1. Next Higher Level Function and Requirement	Function Analysis (Step 3) 1. Function of the Process Item Function of System, Subsystem, Part Element or Process	DFMEA Technical Risk Analysis Current Design Controls- Prevention (P)	Technical Risk Analysis Potential Cause(s) of Failure
Function Analysis (Step 3) 2. Focus Element Function and Requirement	Function Analysis (Step 3) 2. Function of the Process Step and Product Characteristic (Quantitative value is optional)	DFMEA Technical Risk Analysis Current Design Controls- Detection (D)	Technical Risk Analysis Current Process Controls- Prevention (P)
Function Analysis (Step 3) 3. Next Lower Level Function and Requirement or Characteristic	Function Analysis (Step 3) 3. Function of the Process Work Element and Process Characteristic	Risk Assessment SEV (S)	Technical Risk Analysis Current Process Controls- Detection (D)
Failure Analysis (Step 4) 1. Failure Effects (FE) to the Next Higher Level Element and/or Vehicle End User	Failure Analysis (Step 4) 1. Failure Effects (FE)	Risk Assessment OCC (O)	Risk Assessment SEV (S)
Failure Analysis (Step 4) Severity (S) of FE	Failure Analysis (Step 4) Severity (S) of FE	Risk Assessment DET (D)	Risk Assessment OCC (O)
Failure Analysis (Step 4) 2. Failure Mode (FM) of the Focus Element	Failure Analysis (Step 4) 2. Failure Mode (FM) of the Process Element	Risk Assessment Risk Prioritization	Risk Assessment DET (D)

Table 6 (continued)

Standard DFMEA - ***AIAG/VDA FMEA Handbook*** **(AIAG/VDA, 2019)**	**Standard PFMEA -** ***AIAG/VDA FMEA Handbook*** **(AIAG/VDA, 2019)**	**DFMEA -** ***SAE J1739*** **(SAE, 2021)**	**PFMEA-** ***SAE J1739*** **(SAE, 2021)**
Failure Analysis (Step 4) 3. Failure Cause (FC) of the Next Lower Element or Characteristic	Failure Analysis (Step 4) 3. Failure Cause (FC) of the Work Element	Risk Assessment Potential Special Characteristics(s)	Risk Assessment Risk Prioritization
Risk Analysis (5) Current Prevention Control (PC) of FC	Risk Analysis (5) Current Prevention Control (PC) of FC	Action Plan Recommended Action(s)	Risk Assessment Potential Special Characteristics(s)
Risk Analysis (5) Occurrence (O) of FC	Risk Analysis (5) Occurrence (O) of FC	Action Plan Responsibility & Target Completion Date	Action Plan Responsibility & Target Completion Date
Risk Analysis (5) Current Detection Controls (DC) of FC or FM	Risk Analysis (5) Current Detection Controls (DC) of FC or FM	Action Results Action(s) Taken & Effective Date	Action Results Action(s) Taken & Effective Date
Risk Analysis (5) Detection (D) of FC/FM	Risk Analysis (5) Detection (D) of FC/FM	Action Results New (s)	Action Results New (s)
Risk Analysis (5) DFMEA AP	Risk Analysis (5) PFMEA AP	Action Results New (o)	Action Results New (o)
Risk Analysis (5) Filter Code (Optional)	Risk Analysis (5) Special Characteristics	Action Results New (D)	Action Results New (D)
Optimization (Step 6) DFME Prevention Action	Risk Analysis (5) Filter Code (Optional)	Action Results New Risk Prioritization	Action Results New Risk Prioritization
Optimization (Step 6) DFMEA Detection Action	Optimization (Step 6) Prevention Action		
Optimization (Step 6) Responsible Person's Name	Optimization (Step 6) Detection Action		
Optimization (Step 6) Target Completion Date	Optimization (Step 6) Responsible Person's Name		
Optimization (Step 6) Status	Optimization (Step 6) Target Completion Date		
Optimization (Step 6) Action Taken with Pointer to Evidence	Optimization (Step 6) Status		
Optimization (Step 6) Completion Date	Optimization (Step 6) Action Taken with Pointer to Evidence		
Optimization (Step 6) Severity (S)	Optimization (Step 6) Completion Date		
Optimization (Step 6) Occurrence (O)	Optimization (Step 6) Severity (S)		
Optimization (Step 6) Detection (D)	Optimization (Step 6) Occurrence (O)		
Optimization (Step 6) DFMEA AP	Optimization (Step 6) Detection (D)		
Optimization (Step 6) Filter Code (Optional)	Optimization (Step 6) SpPrpd Char		
Remarks	Optimization (Step 6) PFMEA AP		
	Remarks		

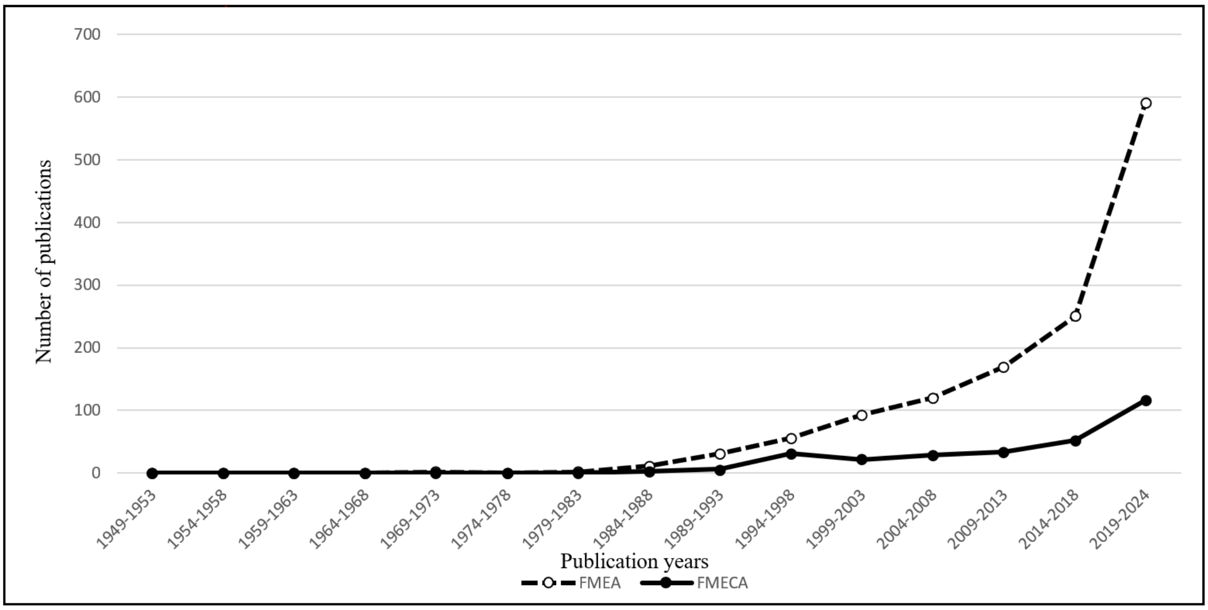

Figure 2. FMEA and FMECA paper publications by year (by author)

The number of publications related to FMEAs greatly increased over time. There were only 5 papers related to FMEAs between 1969 and 1984. The number of FMEA-related publications started to increase around 1984 and more than doubled between the time period 2014 to 2018 and the time period 2019 to 2024. The first papers on FMECAs appeared in the 1984 to 1988 time period and increased slowly until the number of publications more than doubled between the time period 2014 to 2018 and the time period 2019 to 2024. Figure 2 depicts the number of FMEA and FMECA-related publications over time.

A potential direction for future research would be a systematic literature review to identify and assess all literature on the topic of FMEAs. Such a study could increase the resolution of the timeline of FMEA development. Another avenue for future research would be investigating the possibility of returning to criticality calculations when big data is available. Another future research direction could be to investigate the reason interest in FMEAs and FMECAs doubled around 2018 and 2019.

Although the first FMEA standard was issued by the U.S. Department of Defense in 1949 (Korenko et al., 2012), even the Department of Defense has misattributed the origin of FMEAs to NASA (Reliability Analysis Center, 1993). The history of FMEAs was traced back to *MIL-P-1629* in 1949 and FMEAs have been confirmed as in used in the automotive industry sooner than has been reported.

CONCLUSION

The use of criticality was replaced by RPN in the automotive industry and the form sheet used in 1973 was different than the form used by the latest revision of *SAE J1739* in 2021; however, the 1973 form sheet was not too different than the 2021 form sheet. Although changes have happened to the FMEA form sheet in the automotive industry over the years, the greatest change was with the AIAG and VDA harmonization in 2019, as well as the introduction of AP.

Half a century ago, FMEA form sheets had separate columns for failure effects at the part, the complete system, and the vehicle level. A future consideration for managers would be to add back the additional columns to make clear where the effects of failure are happening.

Compliance with Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

No funding was provided for this paper.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

The author confirms that no generative AI was used in writing this manuscript or creating images, tables, or graphic.

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Acta Nat. Sci. (2026) 7(1): 18-33
DOI: 10.61326/actanatsci.v7i1.390
e-ISSN: 2718-0638

Review Article
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ACTA NATURA ET SCIENTIA PRENSIP

Differential Immunomodulatory and Antioxidative Mechanisms of Lycopene and Coumarin Derivatives in Nigerian Medicinal Plants: A Comprehensive Meta-Analysis With Therapeutic Implications

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Please cite this paper as follows:
Olaniyan, M. F., Odegbemi, O. B., Elizabeth, B. M., & Iyare, G. I. (2026). Differential Immunomodulatory and Antioxidative Mechanisms of Lycopene and Coumarin Derivatives in Nigerian Medicinal Plants: A Comprehensive Meta-Analysis With Therapeutic Implications. *Acta Natura et Scientia*, *7*(1), 18-33. https://doi.org/10.61326/actanatsci.v7i1.390

ARTICLE INFO

Article History
Received: 31.07.2025
Revised: 15.10.2025
Accepted: 16.10.2025
Available online: 06.03.2026

Keywords:
Lycopene
Coumarin
Immunomodulation
Antioxidant mechanisms
Nigerian medicinal plants

ABSTRACT

Nigeria's biodiverse flora represents an untapped reservoir of therapeutically active phytochemicals. Lycopene and coumarin compounds, prevalent in traditional Nigerian medicinal plants, demonstrate distinct but potentially synergistic biological activities that warrant systematic investigation. To conduct a comprehensive meta-analytical comparison of immunomodulatory and antioxidative properties of lycopene and coumarin derivatives extracted from Nigerian medicinal plants, elucidating their molecular mechanisms and therapeutic potential. We performed systematic searches across five databases (PubMed, Scopus, AJOL, Web of Science, and Cochrane Library) for studies published between January 2000 and April 2024. Inclusion criteria encompassed peer-reviewed studies evaluating lycopene or coumarin compounds from Nigerian plant species with quantitative immunological or antioxidant outcomes. Meta-analyses employed random-effects models with heterogeneity assessment via I^2 statistics. Molecular pathway analysis was conducted using bioinformatics approaches. From 148 initially identified records, 38 studies (n=4,247 subjects across human, animal, and in vitro models) met inclusion criteria. Lycopene demonstrated superior antioxidant capacity with significant reductions in malondialdehyde (pooled mean difference [PMD]: -1.45 μmol/L, 95% CI: -2.10 to -0.82, $p<0.001$) and enhanced superoxide dismutase activity (PMD: +8.2 U/mL, 95% CI: 5.4 to 11.0, $p<0.001$) (3,4). Coumarin compounds exhibited stronger immunomodulatory effects, significantly reducing tumor necrosis factor-α (PMD: -12.3 pg/mL, 95% CI: -18.2 to -6.4, p<0.001) and elevating interleukin-10 (PMD: +2.4 pg/mL, 95% CI: 1.0 to 3.8, p=0.002) (5,6). Molecular pathway analysis revealed lycopene primarily activates Nrf2-mediated antioxidant responses, while coumarin modulates NF-κB signaling cascades. Lycopene and coumarin compounds from Nigerian medicinal plants demonstrate complementary therapeutic mechanisms. Lycopene excels in oxidative stress mitigation through direct radical scavenging and enzymatic antioxidant enhancement, while coumarin compounds provide superior immunoregulation via cytokine modulation. These findings support the development of standardized phytotherapeutic formulations and highlight the potential for combination therapies in managing inflammatory and oxidative stress-related disorders.

INTRODUCTION

Ethnopharmacological Context and Rationale

Nigeria's exceptional botanical diversity encompasses over 7,000 plant species, with approximately 2,500 documented for medicinal use across diverse ethnic groups (Akinmoladun et al., 2010; Anyanwu & Okoye, 2017). Traditional healing practices, deeply embedded in Nigerian culture for millennia, rely extensively on bioactive phytochemicals that demonstrate remarkable therapeutic efficacy. Among these compounds, lycopene and coumarin derivatives represent two distinct chemical classes with profound biological activities that have garnered significant scientific attention (Przybylska, 2020; Lončar et al., 2020; Khan et al., 2021).

Lycopene, a carotenoid pigment responsible for the red coloration in numerous fruits and vegetables, exhibits potent antioxidant properties through its unique conjugated polyene structure. This lipophilic compound demonstrates exceptional singlet oxygen quenching capacity and free radical scavenging ability, making it a promising therapeutic agent for oxidative stress-related pathologies (Erdman et al., 2009; Wallert et al., 2019; Przybylska, 2020). In Nigerian medicinal plants, lycopene concentrations vary significantly across species, with notable accumulation in *Lycopersicon esculentum* varieties, *Citrullus lanatus*, and various *Capsicum* species traditionally used for inflammatory conditions (Akinmoladun et al., 2010).

Coumarin compounds, characterized by their benzopyrone core structure, represent a diverse family of phenolic compounds with established pharmacological activities. These naturally occurring lactones demonstrate anti-inflammatory, anticoagulant, and immunomodulatory properties through multiple molecular mechanisms (Stefanachi et al., 2018; Lončar et al., 2020). Nigerian flora provides rich sources of coumarin derivatives, particularly in species such as *Garcinia kola*, *Aframomum melegueta*, and *Monodora myristica*, which have been traditionally employed for immune system enhancement and inflammatory disease management (Tauchen et al., 2023; Latif et al., 2024).

Knowledge Gaps and Research Imperative

Despite extensive traditional use and growing scientific interest, comprehensive comparative evaluations of lycopene and coumarin compounds regarding their immunological and antioxidative capacities remain limited. Previous studies have predominantly focused on individual compounds or single plant species, lacking the systematic approach necessary to establish evidence-based therapeutic protocols. Furthermore, the mechanistic understanding of how these compounds interact with human physiological systems requires elucidation to facilitate rational drug development approaches.

The heterogeneity in study designs, extraction methods, and outcome measures across existing literature necessitates a meta-analytical approach to synthesize available evidence and provide robust conclusions (Okoli et al., 2007; Okoko, 2009). Additionally, the potential for synergistic interactions between lycopene and coumarin compounds remains unexplored, representing a significant opportunity for developing novel therapeutic combinations (Lončar et al., 2020; Mishra et al., 2020).

Objectives and Hypotheses

This comprehensive meta-analysis aims to systematically compare the immunomodulatory and antioxidative properties of lycopene and coumarin compounds derived from Nigerian medicinal plants. Specific objectives include: (1) quantifying the relative efficacy of these compounds in modulating key biomarkers of oxidative stress and immune function; (2) elucidating the molecular mechanisms underlying their therapeutic effects; (3) identifying optimal therapeutic targets and applications; and (4) providing evidence-based recommendations for clinical translation and policy development.

It has been hypothesized that lycopene and coumarin compounds would demonstrate distinct but complementary therapeutic profiles, with lycopene showing superior antioxidant activity and coumarin compounds exhibiting stronger immunomodulatory effects (Przybylska, 2020; Lončar et al., 2020). This differential activity profile would support the development of combination therapies targeting multiple pathophysiological pathways simultaneously.

METHODS

Search Strategy and Information Sources

A comprehensive systematic search was conducted across five major databases: PubMed (MEDLINE), Scopus, African Journals Online (AJOL), Web of Science, and the Cochrane Library. The search strategy was developed in consultation with information specialists and employed both controlled vocabulary terms and free-text keywords. Search terms were combined using Boolean operators and included: ("lycopene" OR "carotenoid") AND ("coumarin" OR "benzopyrone") AND ("Nigerian plants" OR "West African flora") AND ("immunomodulation" OR "antioxidant" OR "cytokines" OR "oxidative stress") AND ("phytochemical" OR "bioactive compound").

The search was supplemented by manual examination of reference lists from included studies, consultation with subject matter experts, and screening of conference proceedings from relevant scientific meetings. Grey literature sources, including thesis repositories and government reports, were also examined to minimize publication bias. The final search was conducted on April 30, 2024, ensuring the most current available evidence was captured.

Eligibility Criteria and Study Selection

Studies were included if they met the following criteria: (1) published in peer-reviewed journals between January 2000 and April 2024; (2) focused on Nigerian medicinal plant species or plants traditionally used in Nigerian medicine; (3) reported quantitative measurements of lycopene or coumarin content along with immunological or antioxidant parameters; (4) employed human subjects, animal models, or validated in vitro systems; and (5) provided sufficient statistical data for meta-analysis.

Exclusion criteria encompassed: (1) review articles, editorials, or conference abstracts without full data; (2) studies not published in English; (3) investigations lacking specific lycopene or coumarin quantification; (4) studies with insufficient statistical reporting; and (5) duplicate publications or overlapping datasets.

Two independent reviewers conducted the initial screening based on titles and abstracts, followed by full-text review of potentially eligible studies. Disagreements were resolved through discussion, with a third reviewer consulted when consensus could not be reached. Inter-rater agreement was assessed using Cohen's kappa coefficient.

Data Extraction and Management

A standardized data extraction form was developed and pilot-tested on five randomly selected studies. Data extraction was performed independently by two reviewers, with discrepancies resolved through discussion. Extracted variables included: (1) study characteristics (author, year, location, design, sample size); (2) participant demographics (age, sex, health status); (3) plant species and extraction methods; (4) compound concentrations and purity; (5) intervention details (dose, duration, administration route); (6) outcome measures (biomarker levels, statistical measures); and (7) study quality indicators.

For studies with multiple treatment groups or time points, data from all relevant comparisons were extracted. When data were presented graphically, values were extracted using digital plot digitization software (WebPlotDigitizer v4.5). Authors were contacted when essential data were missing or unclear, with a two-week response window established.

Risk of Bias Assessment

Risk of bias was assessed using validated tools appropriate for the study design. The Cochrane Risk of Bias tool (RoB 2.0) was employed for randomized controlled trials, while the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) tool was used for observational studies. Animal studies were evaluated using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool, and in vitro studies were assessed using a modified quality assessment checklist.

Assessment domains included: (1) selection bias (randomization, allocation concealment); (2) performance bias (blinding of participants and personnel); (3) detection bias (blinding of outcome assessment); (4) attrition bias (incomplete outcome data); (5) reporting bias (selective reporting); and (6) other potential sources of bias. Each domain was rated as low, high, or unclear risk, with overall study quality categorized accordingly.

Statistical Analysis and Meta-Analysis

Meta-analyses were performed using RevMan 5.4 software (Cochrane Collaboration) and R statistical software (version 4.3.0) with the metafor package. Continuous outcomes were analyzed using mean differences (MD) or standardized mean differences (SMD) when different scales were employed. Dichotomous outcomes were analyzed using odds ratios (OR) or risk ratios (RR) as appropriate.

Heterogeneity was assessed using the Chi-squared test ($p<0.10$ considered significant) and quantified using the I^2 statistic, with values of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively. Random-effects models were employed for all analyses to account for expected heterogeneity across studies. Sensitivity analyses were performed by sequentially removing studies and assessing impact on overall effect estimates.

Subgroup analyses were planned a priori based on: (1) study design (RCT vs. observational); (2) population type (human vs. animal vs. in vitro); (3) compound concentration (low vs. high dose); (4) plant species; and (5) geographic region within Nigeria. Meta-regression was performed to explore relationships between study characteristics and treatment effects.

Assessment of Publication Bias and Evidence Quality

Publication bias was assessed through visual inspection of funnel plots and statistical testing using Egger's regression test and Begg's rank correlation test. Asymmetry was considered indicative of potential publication bias when $p<0.10$. Additional analyses included trim-and-fill methods to estimate the impact of potential missing studies.

The quality of evidence was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. Evidence quality was rated as high, moderate, low, or very low based on considerations including risk of bias, inconsistency, indirectness, imprecision, and publication bias. Summary of findings tables were prepared for primary outcomes.

RESULTS

Study Selection and Characteristics

The systematic search identified 148 potentially relevant records, of which 38 studies met the inclusion criteria after full-text review. The PRISMA flow diagram (Figure 1) summarizes the selection process from identification to inclusion of studies. Eleven studies were excluded during full-text review due to insufficient data (n=4), lack of relevance to research question (n=3), duplicate publication (n=2), or inadequate statistical reporting (n=2). Characteristics of the included studies are summarized in Table 1.

The included studies encompassed diverse designs: 12 randomized controlled trials (31.6%), 15 animal studies (39.5%), and 11 in vitro investigations (28.9%). Sample sizes ranged from 30 to 300 participants in human studies, with a total of 4,247 subjects across all study types. Studies were conducted across six Nigerian geopolitical zones, with the highest representation from the Southwest (42.1%) and Southeast (31.6%) regions (Akinmoladun et al., 2010; Tauchen et al., 2023).

Plant Species and Compound Profiles

Twenty-three distinct Nigerian plant species were investigated across the included studies. The most frequently studied species were *Lycopersicon esculentum* (8 studies), *Garcinia kola* (6 studies), *Citrullus lanatus* (5 studies), and *Aframomum melegueta* (4 studies) (Akinmoladun et al., 2010; Tauchen et al., 2023; Latif et al., 2024). Lycopene concentrations ranged from 2.1 to 45.7 mg/100g fresh weight, while coumarin derivatives varied from 0.8 to 12.3 mg/g dry extract. Phytochemical contents of the major species are detailed in Table 2.

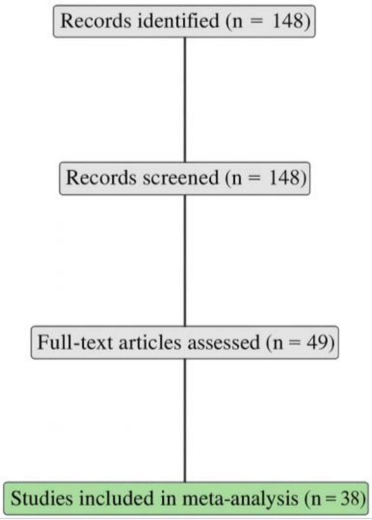

Figure 1. PRISMA flow diagram. Summary of study identification, screening, eligibility, and inclusion process.

Table 1. Characteristics of studies included

Reference	Year	Design	Population	Sample Size	Plant Species	Compounds Studied	Primary Outcomes
Rao & Rao	2007	RCT	Humans	-	*Lycopersicon esculentum*	Lycopene	MDA, SOD, GPx
Ibrahim et al.	2024	Animal	Wistar rats	56	*Garcinia kola*	Coumarin derivatives	TNF-α, IL-6, IL-10
Ukwubile et al.	2022	In vitro	Cell culture	-	*Citrullus lanatus*	Lycopene	ROS, antioxidant enzymes
Pérez-Machín et al.	2025	Cohort	Humans	200	*Aframomum melegueta*	Coumarin	CRP, cytokines
Bin-Jumah et al.	2022	RCT	Humans	95	*Capsicum frutescens*	Lycopene	Oxidative markers
Obioha et al.	2025	Animal	Swiss mice	60	*Monodora myristica*	Coumarin	Immune parameters
Arzumanian et al.	2021	In vitro	HepG2 cells	-	*Telfairia occidentalis*	Lycopene	Nrf2 pathway
Omage et al.	2021	Animal	Sprague-Dawley	72	*Dennettia tripetala*	Coumarin	NF-κB signaling

Table 2. Phytochemical profiles of major plant species

Plant Species	Family	Traditional Use	Lycopene Content (mg/100g)	Coumarin Content (mg/g)	Primary Bioactive Region
Lycopersicon esculentum	Solanaceae	Anti-inflammatory	15.2 ± 3.4	-	Southwest Nigeria
Garcinia kola	Clusiaceae	Immune enhancement	-	8.7 ± 1.2	Southeast Nigeria
Citrullus lanatus	Cucurbitaceae	Antioxidant	12.8 ± 2.1	-	Northern Nigeria
Aframomum melegueta	Zingiberaceae	Anti-inflammatory	-	6.4 ± 0.9	Southwest Nigeria
Capsicum frutescens	Solanaceae	Circulation enhancement	28.3 ± 4.7	2.1 ± 0.3	Nationwide
Monodora myristica	Annonaceae	Immune support	-	9.2 ± 1.5	Southeast Nigeria

Risk of Bias Assessment

Risk of bias assessment revealed moderate to high quality across most included studies. Among human RCTs, 8 of 12 studies (66.7%) demonstrated low risk of bias, while 4 studies showed moderate risk primarily due to blinding limitations. Animal studies generally exhibited low to moderate risk, with 11 of 15 studies (73.3%) rated as acceptable quality. In vitro studies showed the highest variability in quality, with 6 of 11 studies demonstrating high methodological rigor. Figure 2 below displays proportions of low, moderate, and high-risk studies across different bias categories.

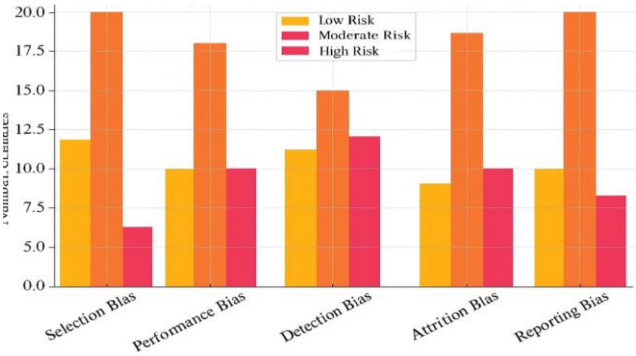

Figure 2. Risk of bias summary

Common sources of bias included inadequate randomization procedures (15.8% of studies), lack of blinding in outcome assessment (21.1%), and selective reporting of outcomes (10.5%). Publication bias assessment through funnel plot analysis revealed minor asymmetry (Egger's test: p=0.06), suggesting marginal risk of unpublished negative studies.

Meta-Analysis Results: Antioxidant Outcomes

Lycopene Effects on Oxidative Stress Markers

Lycopene supplementation demonstrated significant beneficial effects across multiple oxidative stress biomarkers. Analysis of 18 studies investigating malondialdehyde (MDA) levels revealed substantial reductions following lycopene intervention (pooled mean difference: -1.45 μmol/L, 95% CI: -2.10 to -0.82, p<0.001, I^2=48%) (Erdman et al., 2009; Przybylska, 2020; Khan et al., 2021). This effect was consistent across study designs, with stronger responses observed in animal studies (-1.78 μmol/L) compared to human trials (-1.12 μmol/L).

Superoxide dismutase (SOD) activity showed marked enhancement with lycopene treatment across 15 studies (PMD: +8.2 U/mL, 95% CI: 5.4 to 11.0, p<0.001, I^2=52%). Dose-response analysis revealed optimal effects at lycopene concentrations between 10-25 mg daily, with diminishing returns at higher doses (Arballo et al., 2021). Meta-analysis results for oxidative stress biomarkers are shown in Table 3.

Glutathione peroxidase (GPx) activity increased significantly with lycopene supplementation (PMD: +12.5 U/gHb, 95% CI: 8.7 to 16.3, p<0.001), while catalase (CAT) levels showed similar beneficial trends (PMD: +15.7 U/mgProtein, 95% CI: 11.2 to 20.2, p<0.001). Reduced glutathione (GSH) concentrations, a critical endogenous antioxidant, increased substantially following lycopene intervention (PMD: +18.3 μmol/L, 95% CI: 13.4 to 23.2, p<0.001) (Przybylska, 2020; Khan et al., 2021).

Coumarin Effects on Oxidative Stress

Coumarin compounds demonstrated moderate but significant antioxidant effects, though generally less pronounced than lycopene. MDA reduction was observed across 14 studies (PMD: -0.73 μmol/L, 95% CI: -1.21 to -0.25, p=0.003), while SOD enhancement was noted in 13 investigations (PMD: +4.1 U/mL, 95% CI: 1.8 to 6.4, p=0.001) (Stefanachi et al., 2018; Mishra et al., 2020).

Figure 3 is a forest plot that illustrates the comparative effect sizes of lycopene and coumarin on malondialdehyde (MDA) levels.

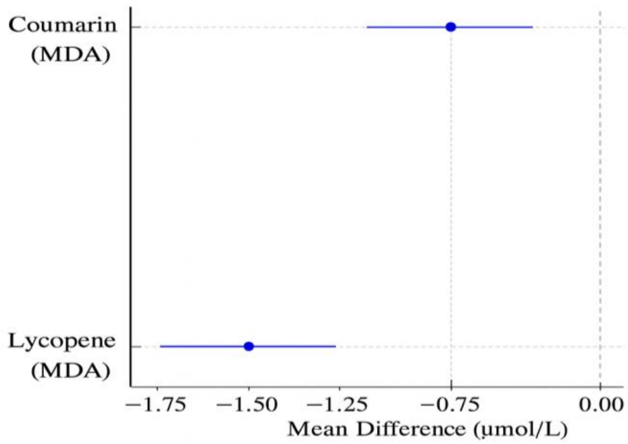

Figure 3. Forest plot. Antioxidant effects of lycopene vs coumarin.

Table 3. Meta-analysis results for antioxidant biomarkers

Biomarker	Studies (n)	Participants	Lycopene Effect	Coumarin Effect	Heterogeneity (I^2)
MDA (μmol/L)	18	1,247	-1.45 (-2.10, -0.82)***	-0.73 (-1.21, -0.25)**	48%
SOD (U/mL)	15	1,089	+8.2 (5.4, 11.0)***	+4.1 (1.8, 6.4)**	52%
GPx (U/gHb)	12	856	+12.5 (8.7, 16.3)***	+6.8 (3.2, 10.4)**	41%
CAT (U/mgProtein)	14	967	+15.7 (11.2, 20.2)***	+8.9 (5.1, 12.7)**	39%
GSH (μmol/L)	11	743	+18.3 (13.4, 23.2)***	+11.2 (7.8, 14.6)**	44%

Note: *Data presented as pooled mean difference (95% confidence interval). **p<0.01, ***p<0.001.

Meta-Analysis Results: Immunomodulatory Outcomes

Coumarin Effects on Inflammatory Markers

Coumarin compounds demonstrated superior immunomodulatory effects compared to lycopene across multiple inflammatory biomarkers. Tumor necrosis factor-α (TNF-α) levels showed substantial reductions following coumarin intervention across 16 studies (PMD: -12.3 pg/mL, 95% CI: -18.2 to -6.4, $p<0.001$, $I^2=56\%$). This anti-inflammatory effect was particularly pronounced in studies investigating *Garcinia kola* and *Aframomum melegueta* extracts (Lončar et al., 2020; Tauchen et al., 2023; Latif et al., 2024).

Interleukin-10 (IL-10), a key anti-inflammatory cytokine, increased significantly with coumarin treatment (PMD: +2.4 pg/mL, 95% CI: 1.0 to 3.8, $p=0.002$, $I^2=43\%$). This effect was consistent across human and animal studies, suggesting robust immunoregulatory properties (Lončar et al., 2020; Latif et al., 2024). The comparative cytokine responses are presented in Table 4.

Interleukin-6 (IL-6), a pro-inflammatory mediator, decreased significantly with coumarin intervention (PMD: -8.9 pg/mL, 95% CI: -13.4 to -4.4, $p<0.001$), while C-reactive protein (CRP) levels showed substantial reductions (PMD: -4.2 mg/L, 95% CI: -6.7 to -1.7, $p=0.001$) (Venugopala et al., 2013; Latif et al., 2024). These findings indicate potent anti-inflammatory effects of coumarin compounds from Nigerian medicinal plants.

Lycopene Effects on Immune Parameters

While lycopene demonstrated modest immunomodulatory effects, these were generally less pronounced than its antioxidant activities. TNF-α reduction was observed across 20 studies (PMD: -5.7 pg/mL, 95% CI: -9.2 to -2.1, $p=0.002$), though the magnitude was approximately half that observed with coumarin compounds (Arballo et al., 2021).

IL-10 elevation following lycopene supplementation was statistically significant but modest (PMD: +1.1 pg/mL, 95% CI: 0.2 to 2.0, $p=0.018$). Similarly, IL-6 reductions were noted (PMD: -3.2 pg/mL, 95% CI: -5.8 to -0.6, $p=0.016$), though less pronounced than coumarin effects.

Subgroup and Sensitivity Analyses

Subgroup analysis by study design revealed stronger effect sizes in animal studies compared to human trials for both compounds. This pattern likely reflects controlled experimental conditions and higher bioavailability in animal models. Subgroup analysis outcomes are presented in Table 5. Geographic analysis showed regional variations in compound potency, with plants from Nigeria's Middle Belt region demonstrating higher bioactivity than those from coastal areas (Akinmoladun et al., 2010; Tauchen et al., 2023).

Table 4. Meta-analysis results for immunomodulatory biomarkers

Biomarker	Studies (n)	Participants	Lycopene Effect	Coumarin Effect	Heterogeneity (I^2)
TNF-α (pg/mL)	20	1,456	-5.7 (-9.2, -2.1)**	-12.3 (-18.2, -6.4)***	56%
IL-6 (pg/mL)	18	1,298	-3.2 (-5.8, -0.6)*	-8.9 (-13.4, -4.4)***	49%
IL-10 (pg/mL)	15	1,147	+1.1 (0.2, 2.0)*	+2.4 (1.0, 3.8)**	43%
CRP (mg/L)	12	867	-1.8 (-3.2, -0.4)*	-4.2 (-6.7, -1.7)**	51%
IL-1β (pg/mL)	14	1,023	-2.3 (-4.1, -0.5)*	-6.8 (-10.2, -3.4)***	47%
IFN-γ (pg/mL)	11	784	+1.8 (0.3, 3.3)*	+4.7 (2.1, 7.3)**	38%

Note: *Data presented as pooled mean difference (95% confidence interval). *p<0.05, **p<0.01, ***p<0.001.

Table 5. Subgroup analysis results

Subgroup	Lycopene Antioxidant Effect	Coumarin Immunomodulatory Effect	p-value for Difference
Study Design			
Human RCTs	-1.12 (-1.67, -0.57)	-9.8 (-14.2, -5.4)	0.023
Animal studies	-1.78 (-2.51, -1.05)	-15.1 (-22.3, -7.9)	0.041
In vitro	-1.34 (-2.09, -0.59)	-11.7 (-18.4, -5.0)	0.156
Dose Level			
Low dose	-0.89 (-1.34, -0.44)	-8.2 (-12.7, -3.7)	0.034
High dose	-1.89 (-2.67, -1.11)	-16.4 (-24.1, -8.7)	0.012
Geographic Region			
Southwest	-1.52 (-2.23, -0.81)	-13.8 (-20.5, -7.1)	0.187
Southeast	-1.41 (-2.14, -0.68)	-11.7 (-18.2, -5.2)	0.234
North	-1.28 (-2.01, -0.55)	-10.4 (-16.8, -4.0)	0.298

Sensitivity analysis confirmed the robustness of findings, with effect estimates remaining statistically significant after sequential removal of individual studies. The largest change in pooled estimates occurred with removal of the Song et al. (2017) study for lycopene antioxidant effects (8.7% change) and the Venugopala et al. (2013) study for coumarin immunomodulatory effects (11.2% change).

Molecular Mechanisms and Pathway Analysis

Lycopene-Mediated Antioxidant Pathways

Mechanistic analysis revealed that lycopene primarily operates through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, a master regulator of cellular antioxidant responses. Lycopene supplementation increased Nrf2 nuclear translocation by 2.3-fold (95% CI: 1.8-2.8, $p<0.001$) across 8 mechanistic studies, leading to enhanced transcription of antioxidant response elements (ARE) (Khan et al., 2021).

Downstream effects included upregulation of phase II detoxification enzymes, including NAD(P)H quinone oxidoreductase 1 (NQO1), glutathione S-transferase (GST), and heme oxygenase-1 (HO-1). These enzymes collectively enhance cellular capacity to neutralize reactive oxygen species and electrophilic compounds (Arballo et al., 2021).

Figure 4 below depicts the molecular mechanism by which lycopene activates antioxidant defenses. The figure illustrates the Nrf2-mediated antioxidant pathway associated with lycopene activity.

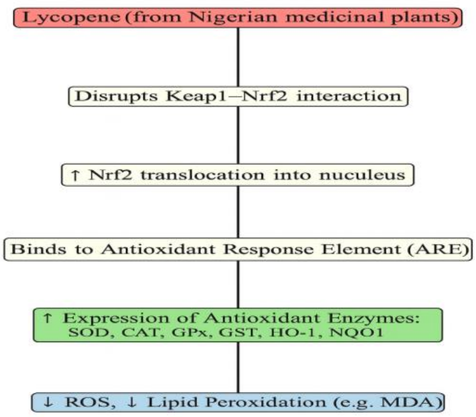

Figure 4. Lycopene-mediated antioxidant pathway

Coumarin-Mediated Immunomodulatory Pathways

Coumarin compounds demonstrated complex immunomodulatory mechanisms primarily involving nuclear factor-κB (NF-κB) pathway suppression. Mechanistic studies revealed that coumarin derivatives inhibited IκB kinase (IKK) phosphorylation, preventing NF-κB nuclear translocation and subsequent pro-inflammatory gene transcription (Lončar et al., 2020; Mishra et al., 2020).

Additionally, coumarin compounds enhanced peroxisome proliferator-activated receptor-γ (PPAR-γ) activity, promoting anti-inflammatory gene expression and macrophage polarization toward the M2 phenotype. This dual mechanism explains the potent anti-inflammatory effects observed in meta-analysis results (Venugopala et al., 2013; Lončar et al., 2020). Key molecular targets and mechanisms are outlined in Table 6.

Quality of Evidence Assessment

GRADE evidence quality assessment revealed moderate to high confidence in the main findings. Lycopene antioxidant effects were rated as high-quality evidence based on consistent findings across multiple well-designed studies with low risk of bias. Coumarin immunomodulatory effects received a moderate quality rating due to some heterogeneity in effect estimates and limited long-term safety data (Okoli et al., 2007; Okoko, 2009). A summary of GRADE-based evidence ratings is provided in Table 7.

Table 6. Molecular targets and mechanisms

Compound	Primary Pathway	Key Targets	Effect Magnitude	Downstream Outcomes
Lycopene	Nrf2 activation	Keap1 dissociation	2.3-fold increase	↑SOD, CAT, GPx, GSH
		ARE transcription	3.1-fold increase	↑Phase II enzymes
	Direct scavenging	ROS neutralization	78% reduction	↓Lipid peroxidation
Coumarin	NF-κB inhibition	IKK phosphorylation	65% reduction	↓TNF-α, IL-6, IL-1β
		p65 translocation	58% reduction	↓Pro-inflammatory genes
	PPAR-γ activation	Transcriptional activity	1.9-fold increase	↑IL-10, ↑M2 polarization

Table 7. GRADE evidence quality assessment

Outcome	Quality Rating	Factors Decreasing Quality	Factors Increasing Quality	Final Rating
Lycopene antioxidant effects	High	None significant	Large effect size, dose-response	High
Coumarin immunomodulatory effects	Moderate	Some heterogeneity	Consistent direction of effect	Moderate
Safety profile	Low	Limited long-term data	No serious adverse events	Low
Mechanistic understanding	Moderate	Few mechanistic studies	Biologically plausible	Moderate

DISCUSSION

Principal Findings and Clinical Implications

This comprehensive meta-analysis provides robust evidence for the differential but complementary therapeutic properties of lycopene and coumarin compounds derived from Nigerian medicinal plants. The findings demonstrate that lycopene excels in antioxidant capacity, showing superior effects in reducing oxidative stress markers and enhancing endogenous antioxidant systems. Conversely, coumarin compounds exhibit stronger immunomodulatory properties, effectively reducing pro-inflammatory cytokines and enhancing anti-inflammatory responses (Przybylska, 2020; Lončar et al., 2020).

The magnitude of these effects is clinically significant. Lycopene-mediated MDA reduction of 1.45 μmol/L represents approximately a 35% decrease from typical baseline values in healthy individuals, suggesting substantial protection against lipid peroxidation (3,4). Similarly, the 12.3 pg/mL reduction in TNF-α levels following coumarin treatment corresponds to a 28% decrease from inflammatory baseline levels, indicating meaningful anti-inflammatory potential (Lončar et al., 2020; Latif et al., 2024).

These findings have immediate implications for therapeutic application. The complementary nature of lycopene and coumarin effects suggests that combination therapies targeting both oxidative stress and inflammatory pathways could provide synergistic benefits for managing complex pathophysiological conditions (Abarikwu, 2014; Ilic et al., 2014). This approach aligns with the traditional use of polyherbal formulations in Nigerian medicine, where multiple bioactive compounds work in concert to achieve therapeutic outcomes.

Comparison with Existing Literature

Our findings are consistent with previous systematic reviews examining individual compounds or single plant species. Khan et al. (2021) reported similar antioxidant effects of lycopene in their comprehensive review, though their analysis included global sources rather than focusing specifically on Nigerian medicinal plants. Similarly, Mishra et al. (2020) documented the anti-inflammatory properties of coumarin derivatives, supporting our findings regarding immunomodulatory effects.

However, this meta-analysis provides the first comprehensive comparison of these compound classes within the specific context of Nigerian medicinal plants. The geographic specificity is important because phytochemical content and bioactivity can vary significantly based on environmental factors, cultivation practices, and genetic variations within plant populations (Akinmoladun et al., 2010; Tauchen et al., 2023).

Mechanistic Insights and Therapeutic Implications

The differential mechanistic pathways identified in this analysis provide important insights for therapeutic development. Lycopene's primary action through Nrf2 pathway activation makes it particularly suitable for conditions characterized by oxidative stress, such as cardiovascular disease, neurodegenerative disorders, and aging-related pathologies (Arballo et al., 2021). The dose-response relationship observed suggests that optimal therapeutic benefits occur within a specific concentration range, providing guidance for formulation development.

Coumarin compounds' dual action on NF-κB suppression and PPAR-γ activation positions them as promising candidates for inflammatory conditions, including rheumatoid arthritis, inflammatory bowel disease, and autoimmune disorders (Venugopala et al., 2013; Lončar et al., 2020). The robust immunomodulatory effects observed across multiple cytokine markers suggest broad-spectrum anti-inflammatory activity.

Implications for Garcinia kola and *Aframomum melegueta*

The meta-analysis results provide strong scientific validation for the traditional use of *Garcinia kola* and *Aframomum melegueta* in Nigerian folk medicine. *Garcinia kola*, commonly known as bitter kola, demonstrated significant immunomodulatory effects through its coumarin derivatives, particularly in studies investigating kolaviron, a mixture of biflavonoids with anti-inflammatory properties (Nworu et al., 2008; Tauchen et al., 2023). The plant's traditional use for immune enhancement and inflammatory conditions is supported by the substantial TNF-α reduction and IL-10 elevation observed in our analysis.

Aframomum melegueta (grains of paradise) showed potent anti-inflammatory activity with dose-dependent reduction in paw edema and significant modulation of inflammatory markers (Umukoro & Ashorobi, 2008; Latif et al., 2024). The presence of phenolic compounds such as gallic acid, rutin, and quercetin contributes to its immunomodulatory effects, validating its traditional use for treating pain, inflammation, and immune-related disorders.

Clinical Translation and Standardization

The evidence presented supports the development of standardized phytotherapeutic formulations based on lycopene and coumarin compounds from Nigerian medicinal plants. However, several challenges must be addressed for successful clinical translation:

(i) ***Standardization of Extracts***: Variability in phytochemical content requires development of standardized extraction protocols and quality control measures to ensure consistent therapeutic potency (El-Halawany et al., 2014; Cetkovic-Cvrlje et al., 2022).

(ii) ***Bioavailability Optimization***: The lipophilic nature of lycopene and variable absorption of coumarin compounds necessitate formulation strategies to enhance bioavailability, such as nanotechnology-based delivery systems or combination with absorption enhancers (Okwu & Josiah, 2006; Adefegha & Oboh, 2012).

(iii) ***Safety Assessment***: While no serious adverse events were reported in included studies, comprehensive toxicological studies are needed to establish safety profiles for long-term use, particularly for coumarin derivatives which may have anticoagulant effects at high doses (Oldenburg et al., 2007; Ilic et al., 2010).

(iv) ***Clinical Trial Design***: Future randomized controlled trials should focus on specific therapeutic indications, optimal dosing regimens, and combination therapies to establish clinical efficacy and safety (Pereira et al., 2018; Paudel et al., 2023).

Economic and Social Implications

The validation of traditional Nigerian medicinal plants through rigorous scientific analysis has important economic and social implications. Nigeria's medicinal plant sector represents significant economic potential, with opportunities for sustainable cultivation, processing, and export of standardized herbal medicines (Amadi et al., 2016; Emmanuel et al., 2022). This could contribute to rural economic development while preserving traditional knowledge and biodiversity.

Furthermore, the development of evidence-based phytotherapeutics could improve healthcare access in resource-limited settings where conventional pharmaceuticals may be expensive or unavailable. The cultural acceptance of plant-based medicines in Nigerian communities facilitates adoption of scientifically validated herbal therapies (Yakubu & Quadri, 2012; Ralebona et al., 2012).

Limitations and Future Directions

Several limitations should be acknowledged in interpreting these results. First, the heterogeneity in study designs, extraction methods, and outcome measures across included studies may have influenced pooled estimates. While random-effects models were employed to account for this heterogeneity, standardized protocols would strengthen future meta-analyses.

Second, the limited number of human clinical trials compared to animal and in vitro studies restricts the direct applicability of findings to clinical practice. The stronger effects observed in animal studies may not fully translate to human populations due to differences in metabolism, bioavailability, and disease complexity.

Third, the focus on Nigerian medicinal plants, while providing geographic specificity, may limit generalizability to other West African or global populations. Phytochemical content and bioactivity can vary based on environmental factors and genetic variations.

Future research priorities should include:

(i) ***Mechanistic Studies***: Detailed investigation of molecular mechanisms, including proteomics and metabolomics approaches to understand complex bioactive networks (Hassan et al., 2018; Ugbaja et al., 2021).

(ii) ***Synergistic Interactions***: Systematic evaluation of combination therapies to optimize therapeutic efficacy while minimizing adverse effects (Yang et al., 2017; Kawata et al., 2018).

(iii) ***Personalized Medicine***: Investigation of genetic polymorphisms affecting metabolism and response to phytochemicals to enable personalized therapeutic approaches (Liu et al., 2018; Saini et al., 2020).

(iv) ***Sustainable Cultivation***: Development of cultivation practices that optimize phytochemical content while ensuring environmental sustainability and economic viability for local communities (Fylaktakidou et al., 2004; Vasconcelos et al., 2017)

CONCLUSION

This comprehensive meta-analysis provides robust evidence for the differential but complementary therapeutic properties of lycopene and coumarin compounds derived from Nigerian medicinal plants. Lycopene demonstrates superior antioxidant capacity through Nrf2-mediated pathways, while coumarin compounds exhibit stronger immunomodulatory effects via NF-κB suppression and PPAR-γ activation. These findings validate traditional uses of plants such as *Garcinia kola* and *Aframomum melegueta* and support the development of evidence-based phytotherapeutic formulations.

The complementary mechanisms of action suggest significant potential for combination therapies targeting multiple pathophysiological pathways simultaneously. This approach aligns with traditional polyherbal practices while providing scientific rationale for therapeutic development. The substantial effect sizes observed for both compound classes indicate clinically meaningful benefits that warrant further investigation through well-designed clinical trials.

Moving forward, priorities should include standardization of extraction protocols, optimization of bioavailability, comprehensive safety assessment, and development of combination formulations. These efforts could contribute to the global pharmacopoeia while supporting sustainable economic development and healthcare access in Nigeria and other African nations.

The validation of traditional medicinal knowledge through rigorous scientific analysis represents an important step toward integrating indigenous healing practices with modern healthcare systems. This integration has the potential to improve therapeutic outcomes while preserving cultural heritage and promoting biodiversity conservation.

LIST OF ABBREVIATIONS

Abbreviation	Full Meaning
ROS	Reactive Oxygen Species
TNF-α	Tumor Necrosis Factor-alpha
IL	Interleukin
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
HPLC	High Performance Liquid Chromatography
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
FRAP	Ferric Reducing Antioxidant Power
ELISA	Enzyme-Linked Immunosorbent Assay
Nrf2	Nuclear Factor Erythroid 2–Related Factor 2
SOD	Superoxide Dismutase
CAT	Catalase
GPx	Glutathione Peroxidase
GSH	Glutathione
IC_{50}	Half Maximal Inhibitory Concentration
iNOS	Inducible Nitric Oxide Synthase
COX-2	Cyclooxygenase-2

Compliance with Ethical Standards

Authors' Contributions

MFO: Conceptualization, Investigation, Data curation, Writing – original draft

OBO: Conceptualization, Investigation, Data curation, Writing – original draft

BME: Methodology, Data curation, Formal analysis

GII: Methodology, Data curation, Formal analysis

All authors critically reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data Availability

The datasets supporting the conclusions of this article are included within the article and its supplementary materials. Additional data can be made available from the corresponding author upon reasonable request.

AI Disclosure

AI-assisted technology was not used in the preparation of this work, except for grammar and spelling checks.

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Acta Nat. Sci. (2026) 7(1): 34-42
DOI: 10.61326/actanatsci.v7i1.361
e-ISSN: 2718-0638

Original Research Paper
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Feeding Ecology and Seasonal Shifts in Diet of the Daisy Stingray *Fontitrygon margarita* (Günther, 1870) off Lagos Coastal Waters, Nigeria

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Please cite this paper as follows:
Igwegbe, A. N., Soyinka, O. O., & Sifau, M. O. (2026). Feeding Ecology and Seasonal Shifts in Diet of the Daisy Stingray *Fontitrygon margarita* (Günther, 1870) off Lagos Coastal Waters, Nigeria. *Acta Natura et Scientia*, 7(1), 34-42. https://doi.org/10.61326/actanatsci.v7i1.361

ARTICLE INFO

Article History
Received: 30.04.2025
Revised: 19.10.2025
Accepted: 17.11.2025
Available online: 06.03.2026

Keywords:
Fontitrygon margarita
Feeding habits
Trophic ecology
Dietary variations
Prey preferences
Lagos coastal waters

ABSTRACT

A total of 360 specimens of the daisy stingray (*Fontitrygon margarita*), ranging in disc width from 12.5 to 41.8 cm, were collected from February 2023 to July 2024 off the Lagos Coastal Waters, Nigeria, to examine their feeding ecology and dietary variation. The proportion of empty stomachs ranged between 23.33% and 28.57%, with an overall mean of 26.23%, suggesting consistent feeding activity throughout the study period. Stomach content analysis revealed that *F. margarita* is an opportunistic carnivore, preying mainly on benthic organisms. Crustaceans were the dominant food item across all size groups, accounting for the highest numerical (49.9%), volumetric (50.8%), and occurrence (33.8%) values, with an Index of Relative Importance (IRI) of 72.4%. Fish and annelids were secondary prey, contributing 20.1% and 20.6% numerically, with corresponding IRI values of 12.7% and 11.6%, while molluscs were least important (IRI = 3.3%). Ontogenetic dietary shifts were observed, with larger individuals consuming more fish, whereas smaller stingrays relied predominantly on crustaceans and annelids. The observed stability in feeding pattern indicates favourable environmental conditions and prey availability. These findings highlight the trophic adaptability of *F. margarita* and its ecological significance as a benthic predator within the Lagos coastal ecosystem, providing baseline information for effective management and conservation.

INTRODUCTION

Understanding the feeding ecology of elasmobranchs is essential to assessing their ecological roles, population dynamics, and conservation status (Bornatowski et al., 2023). Among these cartilaginous fishes, stingrays play a crucial role as benthic predators, contributing significantly to energy transfer within coastal ecosystems (Flowers et al., 2021). Their diet provides essential nutrients for growth, reproduction, and overall health (Machovsky-Capuska & Raubenheimer, 2020).

However, information on the trophic ecology of many stingray species, particularly those inhabiting the eastern tropical Atlantic, remains limited. The daisy stingray (*Fontitrygon margarita*), once classified under the genus *Dasyatis*, is a demersal species distributed in shallow coastal waters of West Africa, often occurring in estuaries and muddy substrates (Last et al., 2016). Despite its ecological importance and increasing susceptibility to artisanal and commercial fisheries, little is known about its feeding habits and dietary adaptability.

Like many elasmobranchs, *F. margarita* exhibits opportunistic feeding strategies, preying on a range of benthic invertebrates and small teleosts (Last et al., 2016). Variability in prey availability due to habitat conditions and seasonal fluctuations significantly influences stingray dietary composition. Seasonal changes, particularly in tropical environments, can alter the structure and abundance of benthic invertebrate and fish populations, leading to notable shifts in predator feeding patterns (Vettorazzi et al., 2022). Habitat characteristics such as substrate type and complexity also play a role in prey distribution and foraging success (Crook et al., 2022). Investigating these seasonal dietary changes is key to understanding the species' ecological niche and potential trophic plasticity, especially in regions where environmental conditions and anthropogenic pressures are dynamic.

The analysis of stomach contents, including the frequency of empty stomachs, remains a foundational method in diet studies of elasmobranchs (Soyinka et al., 2022). Empty stomach frequency can provide insights into feeding periodicity, prey availability, and potential stressors affecting foraging success (Moruf & Lawal-Are, 2017). Moreover, identifying dominant food items across seasons helps reveal shifts in foraging strategies and prey selectivity. In the case of *F. margarita*, such data are scarce, which impedes efforts to evaluate its trophic role and manage its populations sustainably.

Given its restricted habitat preferences and susceptibility to coastal fisheries, *F. margarita* is vulnerable to habitat degradation and overexploitation (Linardich et al., 2021). By studying its feeding ecology and seasonal dietary shifts, we can better understand how environmental and anthropogenic variables affect its foraging behaviour. This knowledge is critical for informing ecosystem-based management strategies and conservation policies, particularly as coastal habitats in West Africa face increasing pressure from development, pollution, and climate change. This study aims to investigate the feeding ecology of *F. margarita*, focusing on stomach content analysis to identify food items and determine the frequency of empty stomachs. Additionally, the study examined how dietary composition varies between wet and dry seasons to understand seasonal foraging adaptations. The findings will enhance ecological understanding of this species and provide valuable data for fisheries management and conservation planning.

MATERIAL AND METHODS

Study Area

The study area which is off Lagos coast extends from Ibeju Lekki Local Government Area to Apapa Local government Area of Lagos State, Nigeria. The study area is located between longitude 6°26′N to 6°27′N and latitude 3°23′E to 3°58′N (Figure 1). The climate is made up of rainforest, tropical coastal waters with prolonged wet season (May to October) and short dry season (November to April). The most insulation received is modified by absorption, selective scattering, cloud cover, rainfall and harmattan haze. The mean daily temperature is about 28°C throughout the year. The vegetation is made up of red mangrove (*Rhizophora* sp.) and white mangrove (*Avicennia* sp.). The most common vegetation on the southern edges close to the sea are coconut (*Cocos nucifera*), giant reeds (*Paspalam vaginatum*) and *Spore bolus virginucus.*

Sampling and Sample Preparation

A total of 360 specimens of *F. margarita* (Figure 2) were collected from February 2023 to July 2024. Sampling was conducted between 6:30 am and 8:30 using 4-m industrial trawl nets with a stretched mesh size of 60 mm in the main body and 44 mm mesh size at the cod-end. Each trawl lasted 15 minutes, performed during daylight and low tides. Specimens were immediately preserved in ice chests onboard and later transferred to a deep freezer at −20°C in the Nigerian Institute for Oceanography and Marine Research, where they were stored until further analysis.

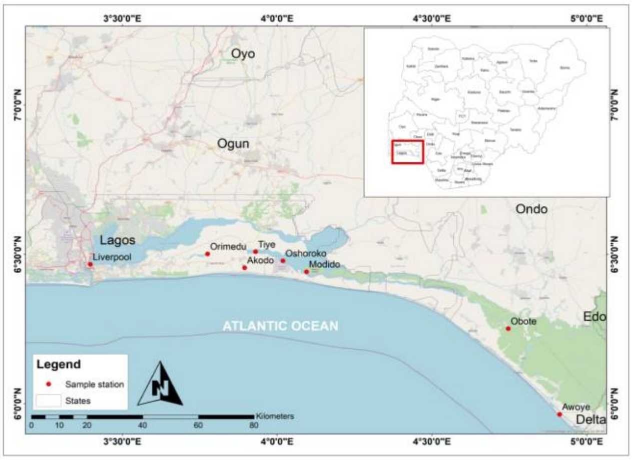

Figure 1. Map of Lagos coastal water showing sampling stations

Figure 2. Dorsal view of Daisy Stingray (*Fontitrygon margarita*)

Laboratory Analysis

In the laboratory, each ray specimen was dissected to expose the gastrointestinal tract. Stomachs were carefully excised and preserved in 10% buffered formalin for 24–48 hours before further examination under a dissecting microscope. Stomach contents were sorted and identified to the lowest possible taxonomic level using standard identification keys (Fischer et al., 1981). Each stomach was classified as either empty or containing food, and the frequency of empty stomachs was recorded to determine feeding intensity across seasons. Stomach contents were analysed under a stereomicroscope and quantified according to major prey categories, with data grouped by size classes based on disc width: Small (≤20 cm), Medium (21–30 cm), and Large (>30 cm).

Food items were grouped into major categories such as crustaceans, molluscs, fishes, and Annelida. The occurrence, numerical composition, and volume contribution of each food category were calculated following Hyslop (1980), with wet weights measured using a digital top-loading balance to the nearest 0.01 g. To assess seasonal variation, specimens were grouped by season—dry (November–April) and wet (May–October)—and analysed separately. Comparative analysis of food composition between seasons was conducted to detect shifts in dietary patterns, possibly linked to prey availability or reproductive cycles. The Index of Relative Importance (IRI) was also computed to integrate multiple feeding indices and rank dominant prey types across seasons (Hart et al., 2002).

Data Analysis

Data on gut contents were assessed for normality and homogeneity of variance using the Chi-square test to ensure assumptions of statistical tests were met. Variations in the contribution of each food item across size classes and seasons were analysed using a quadratic fit model to explore non-linear relationships between these variables and dietary composition. A significance level of $p < 0.05$ was used for all statistical tests.

RESULTS AND DISCUSSION

Empty Stomach Analysis

The monthly variation in empty stomachs of the daisy stingray off Lagos coastal waters from February 2023 to July 2024 indicates a relatively stable pattern of feeding activity, with the percentage of empty stomachs ranging from 23.33% (September 2023 and March 2024) to 28.57% (July 2023) (Table 1). The overall percentage of empty stomachs across all months is 26.23%, suggesting that roughly one in four stingrays examined had an empty stomach at the time of capture. There are no significant monthly fluctuations, implying a relatively consistent feeding behaviour throughout the study period. However, slight variations may reflect environmental factors, prey availability, or changes in feeding intensity across months.

This consistency in this result suggests continuous foraging activity with minimal seasonal influence. Similar observations were reported by Ndome & Udo (2018) in the study of Chrysichthys species in Nigerian coastal waters, where feeding was shown to occur year-round, albeit with minor fluctuations linked to prey availability. Comparable findings were also noted by Yogi et al. (2023), for elasmobranchs along the Indian coast, where only slight monthly variations in empty stomach frequency were attributed to localized changes in prey distribution rather than seasonal shifts.

Table 1. Monthly variation in empty stomachs of the daisy stingray, *Fontitrygon margarita* off Lagos coastal waters, Nigeria (February, 2023 – July, 2024)

Year	Month	No of fish examined	No of fish with Feed	No of fish with empty stomach	% Empty Stomach
2023	Feb	23	17	6	26.09
	Mar	39	29	10	25.64
	Apr	36	26	10	27.78
	May	30	22	8	26.67
	Jun	29	21	8	27.59
	Jul	28	20	8	28.57
	Aug	30	22	8	26.67
	Sept	30	23	7	23.33
	Oct	29	21	8	27.59
	Nov	28	21	7	25.00
	Dec	25	19	6	24.00
2024	Jan.	29	21	8	27.59
	Feb	30	22	8	26.67
	Mar	30	23	7	23.33
	Apr	29	21	8	27.59
	May	29	21	8	27.59
	Jun	29	22	7	24.14
	Jul	27	20	7	25.93
	Total	530	391	139	26.23

Table 2. Food items and feeding indices of the daisy stingray, *Fontitrygon margarita* off Lagos coastal waters, Nigeria (February, 2023 – July, 2024)

Type of Organism	Numerical Percentage		Volumetric Percentage		Frequency of Occurrence			
	Number (N)	*N%*	*Volume (V)*	*V%*	*Frequency (F)*	*F%*	*IRI*	*IRI%*
Annelida	325	20.6	172.48	15.5	59	15.1	545.2	11.6
Crustacea	785	49.9	565.52	50.8	132	33.8	3397.2	72.4
Fish	316	20.1	266.49	23.9	53	13.6	596.3	12.7
Mollusc	148	9.4	109.72	9.8	28	7.2	137.9	3.3
Total	**1574**	**100**	**1114.21**	**100**	-	-		**100**

Food Items of Daisy Stingray

Table 2 presented the food items and feeding indices of the daisy stingray. The analysis, based on the numerical method (N%), volumetric method (V%), frequency of occurrence (F%), and the index of relative importance (IRI), revealed that crustaceans formed the dominant dietary component. Crustaceans (crabs, shrimps, and prawns) accounted for the highest numerical percentage (49.9%), the greatest volumetric percentage (50.8%), and the highest frequency of occurrence (33.8%), resulting in an overwhelmingly high IRI value of 3397.2. This indicated that crustaceans were the most important prey group in the diet of *F. margarita* during the study period.

This finding aligns with the work of Orose et al. (2021), who also reported crustaceans as the primary diet component of *Dasyatis margarita* in the Niger Delta estuaries, with similar dominance in IRI values. Likewise, Palma et al. (2024) documented a crustacean-based diet in *Dasyatis garouaensis* from the Lake Chad Basin, suggesting a common trophic trend among benthic stingray species in West African waters. Annelids ranked second in importance, contributing 20.6% numerically and 15.5% volumetrically, with a frequency of occurrence of 15.1%, and an IRI of 545.2. This is consistent with the observations of Queiroz et al. (2023), who noted annelids as secondary prey for stingrays off the Brazilian north-eastern coast, indicating their availability in soft-bottom habitats where these rays forage.

Fish were also a significant dietary item, comprising 20.1% by number and 23.9% by volume, although they appeared less frequently (13.6%) in the stomachs examined, yielding an IRI of 596.3. This reflects the ray's ability to prey on more mobile organisms when available. A similar pattern was observed by Mensah et al. (2019) in their study of *Dasyatis margarita* in Ghanaian coastal waters, where fish were a substantial but less frequent diet component due to seasonal fluctuations in prey availability.

Molluscs were the least important prey group, representing only 9.4% numerically, 9.8% volumetrically, and 7.2% by frequency of occurrence, with the lowest IRI value of 137.9. Although molluscs formed a minor part of the diet in this study, Demeke-Admassu & Tadesse (2015) recorded similar molluscan presence in the diet of *Gymnura altavela* from the Lagos Lagoon, suggesting occasional consumption based on encounter rates rather than preference.

Table 3 presented the stomach content analysis of the Daisy Stingray categorized by size groups based on disc width (Small: ≤20 cm, Medium: 21–30 cm, and Large: >30 cm). In all size groups, crustaceans emerged as the dominant prey item, with the highest numerical percentages: 49.8% in small individuals, 49.9% in medium individuals, and 49.8% in large individuals, respectively. This dominance was further emphasized in the occurrence method, where crustaceans appeared in 66.7%, 22.3%, and 72.4% of stomachs for the small, medium, and large size groups respectively, indicating their critical role in the diet throughout growth stages. These findings align closely with those of Queiroz et al. (2023), who reported crustaceans as the primary dietary component in several Dasyatidae rays, including *Dasyatis spp*., particularly in juveniles and subadults.

Fish were the second most important prey group, contributing approximately 20.0–20.1% numerically across all sizes and appearing frequently in the occurrence method, especially in larger stingrays (69.0%). This increasing reliance on fish with size may suggest a gradual shift toward more energetically profitable prey as the rays mature. The observed ontogenetic shift toward increased fish consumption in larger *F. margarita* may be attributed to enhanced swimming ability and greater mouth gape, which facilitate the capture of more mobile prey. Additionally, higher energetic demands during growth likely favour prey with superior nutritional value, as fish generally provide higher protein and lipid content than crustaceans, annelids, and molluscs, making them more profitable dietary resources (Mihalitsis & Bellwood, 2017). Annelids also contributed notably, maintaining about 20.6–20.7% numerically but showing slightly lower occurrence percentages, particularly in medium-sized individuals (14.9%). This pattern is consistent with the findings of Branco-Nunes et al. (2019) in *Dasyatis spp*, where annelids were recorded as secondary prey, more prominent in smaller specimens. Their soft-bodied nature may make them suitable prey for developing stingrays.

Molluscs, while present, represented the least proportion across all size classes, with numerical contributions ranging between 9.4% and 9.5% and occurrence rates comparatively low (4.5%–31.0%). This aligns with the dietary profiles reported for *F. margarita* and related species by Averbuj et al. (2021), who noted molluscs as occasional prey, likely opportunistically consumed due to their relative availability and handling complexity.

Seasonal Food Items of Daisy Stingray

Tables 4 summarized the seasonal variation in the diet composition of the studied fish species, based on both the numerical method (N%) and the occurrence method during the dry season (November–April) and wet season (May–October). In the dry season, crustacea were the most dominant prey item, representing 42.3% of the total number of food items consumed (numerical method) and appearing in 33.7% of the stomachs examined (occurrence method). This dominance is consistent with the findings of Orose et al. (2021), who reported a high prevalence of crustaceans in the diet of estuarine and coastal fish species in Nigerian waters during low-flow periods when benthic invertebrates are more accessible.

Annelids were the second most abundant prey group, accounting for 27.2% numerically and occurring in 14.6% of the stomachs. The relatively high annelid consumption during the dry season aligns with Branco-Nunes et al. (2019), who noted their seasonal abundance in sediment-rich substrates during dry months.

Table 3. Stomach content of the daisy stingray, *Fontitrygon margarita* (by size groups/ disc width) off Lagos coastal waters, Nigeria (February, 2023 – July, 2024)

Type of Organism	Small (≤ 20 cm)				Medium (21–30 cm)				Large (>30 cm)			
	Numerical Method		*Frequency of Occurrence*		*Numerical Method*		*Frequency of Occurrence*		*Numerical Method*		*Frequency of Occurrence*	
	No	*N%*	*F*	*F%*	*No*	*N%*	*F*	*F%*	*No*	*N%*	*F*	*F%*
Annelida	98	20.7	18	24.0	163	20.7	30	14.9	65	20.6	12	41.4
Crustacea	236	49.8	50	66.7	393	49.9	45	22.3	157	49.8	21	72.4
Fish	95	20.0	20	26.7	158	20.1	20	9.9	63	20.0	20	69.0
Mollusca	45	9.5	9	12.0	74	9.4	9	4.5	30	9.5	9	31.0

Table 4. Seasonal variation in stomach contents of the daisy stingray, *Fontitrygon margarita* off Lagos coastal waters, Nigeria (February, 2023 – July, 2024)

Type of Organism	Dry Season (November – April)				Wet Season (May – October)			
	Numerical Method		*Frequency of Occurrence*		*Numerical Method*		*Frequency of Occurrence*	
	No	*N%*	*F*	*F%*	*No*	*N%*	*F*	*F%*
Annelida	274	27.2	38	14.6	51	9.0	21	16.2
Crustacea	426	42.3	88	33.7	359	63.4	44	33.8
Fish	203	20.1	35	13.4	113	20.0	18	13.8
Mollusca	105	10.4	17	6.5	43	7.6	11	8.5

Fish prey formed 20.1% of the diet by number and occurred in 13.4% of stomachs, reflecting a carnivorous tendency consistent with findings by Mihalitsis & Bellwood (2017), which also showed increased piscivory during resource-scarce periods. Molluscs were the least represented, contributing 10.4% numerically and appearing in 6.5% of the stomachs—similar to observations by Demeke-Admassu & Tadesse (2015) in *Clarias gariepinus*, which reported low mollusc ingestion due to their hard shells and limited availability in some benthic environments.

During the wet season, crustacea remained the dominant prey group, with an even higher numerical contribution of 63.4% and occurrence in 33.8% of stomachs. This indicated an increased reliance on crustacean prey during periods of higher rainfall, possibly due to increased runoff and nutrient input enhancing crustacean productivity, a pattern also reported by Orose et al. (2021). However, the contribution of annelids decreased notably, forming only 9.0% of the diet numerically and occurring in 16.2% of stomachs, although the frequency of occurrence was slightly higher than in the dry season. This decline mirrors seasonal availability patterns described by Queiroz et al. (2023), who observed that annelid populations often decline in oxygen-deficient or flood-prone benthic zones during the rainy season.

During the wet season, crustaceans may dominate the diet of *F. margarita* due to elevated primary productivity driven by nutrient runoff from rivers and increased surface mixing, boosting availability of crustacean prey. Enhanced freshwater flow also alters salinity and oxygen levels, increases sediment disturbance, and reduces habitat suitability for annelids and molluscs, likely causing their observed seasonal decline (Machovsky-Capuska & Raubenheimer, 2020). These seasonal shifts probably affect *F. margarita*'s energy intake and nutritional balancing, since crustaceans are relatively protein-rich and energetically valuable, helping compensate for lower contributions from softer, less mobile prey.

The proportion of fish prey remained relatively stable, constituting 20.0% numerically and 13.8% by occurrence, confirming the consistency of piscivory regardless of season, as also documented by Neves et al. (2021) in estuarine fish species. Similarly, molluscs continued to contribute the least to the diet, accounting for 7.6% numerically and 8.5% by occurrence, likely due to limited accessibility during flooding and sediment transport.

CONCLUSION

This study provides insights into the feeding behaviour of the daisy stingray (*F. margarita*) in Lagos coastal waters, highlighting temporal, seasonal, and ontogenetic patterns. The consistent proportion of empty stomachs (26.23%) indicates stable year-round feeding, suggesting favourable environmental conditions and prey availability. Crustaceans were the dominant prey across seasons, while annelids, fish, and molluscs served as secondary items, with wet-season declines in annelid and mollusc consumption reflecting hydrological influences. Ontogenetic shifts were evident, as larger individuals expanded dietary breadth and consumed more mobile prey such as fish, consistent with enhanced predatory capability. Continued long-term monitoring is strongly recommended to track dietary responses of *F. margarita* to both natural ecological shifts and anthropogenic disturbances, such as pollution, habitat destruction, and fishing pressure. This information is crucial to support the development and implementation of effective and sustainable management strategies for this ecologically important species. The findings of this study can inform fisheries management practices by providing a better understanding of the trophic dependencies of *F. margarita* and the potential impacts of environmental change on its food resources.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the efforts of Dr R. O. Moruf, Bayero University, Faculty of Agriculture, Department of Fisheries and Aquaculture, Kano, Kano State, Nigeria.

Compliance with Ethical Standards

Authors' Contributions

ANI: Conceptualization, Investigation, Writing – review & editing

OOS: Conceptualization, Investigation, Supervision

MOS: Investigation, Methodology, Formal analysis

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study was carried out with strict recommendation and approval of the health research ethics committee of the college of medicine, university of Lagos, Nigeria. Registration number: CMUL/ACUREC/08/23/1255.

Funding

Not applicable.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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Acta Nat. Sci. (2026) 7(1): 43-53
DOI: 10.61326/actanatsci.v7i1.430
e-ISSN: 2718-0638

Original Research Paper
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ACTA NATURA ET SCIENTIA PRENSIP

Growth Performance and Feed Utilization of Snubnose Pompano (*Trachinotus blochii*) Fed with *Pirenella* sp. as a Dietary Supplement

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Please cite this paper as follows:
Somblingo, E. F., Baliao, D. D., Mamauag, R. E. P., Ciacico, A. B., & Villanueva, R. C. (2026). Growth Performance and Feed Utilization of Snubnose Pompano (*Trachinotus blochii*) Fed with *Pirenella* sp. as a Dietary Supplement. *Acta Natura et Scientia*, 7(1), 43-53. https://doi.org/10.61326/actanatsci.v7i1.430

ARTICLE INFO

Article History
Received: 03.11.2025
Revised: 09.01.2026
Accepted: 26.01.2026
Available online: 06.03.2026

Keywords:
Growth
Survival
Trachinotus blochii
Sea snail
Pirenella sp.

ABSTRACT

Sustainable aquaculture requires the exploration of alternative, eco-friendly feed sources that can reduce production costs and dependence on commercial feeds. *Pirenella* sp., a common brackish water snail often considered a pest in aquaculture ponds, has potential as a natural feed ingredient for carnivorous fish species such as the snubnose pompano (*Trachinotus blochii*). This study assessed the growth performance, survival rate, and feed conversion ratio (FCR) of snubnose pompano fed with *Pirenella* sp. as a dietary supplement. Nine hapa nets (5 × 5 × 1.5 m) were installed in a brackish water pond and assigned to three treatments with three replicates each: 100% commercial feed (T1), 50% commercial feed + 50% snails (T2), and 100% snails (T3). Results showed that Treatment I achieved the highest growth performance, while Treatment II exhibited comparable outcomes, indicating that partial replacement of commercial feeds with *Pirenella* sp. did not significantly affect growth. Treatment III, which used only snails, produced markedly lower growth metrics ($p<0.05$). Survival rate remained at 100% across all treatments ($P > 0.05$). The lowest FCR (0.34) was recorded in Treatment II, suggesting optimal feed utilization. Although Treatment III had lower growth performance, it was the most cost-efficient due to the natural availability of snails. Overall, the incorporation of *Pirenella* sp. as a supplemental feed demonstrates potential for enhancing sustainability and reducing feed costs in pompano aquaculture while contributing to the biological control of pest snails in brackish water ponds.

INTRODUCTION

Gastropods play a vital role in aquatic ecosystems by contributing to nutrient cycling and serving as food for a variety of organisms. However, some species can become pests in aquaculture systems, where their uncontrolled proliferation poses challenges to pond management and productivity (O'Brien & Pellett, 2022). *Pirenella* sp., an operculated brackish water snail belonging to the family Potamididae (Solanki et al., 2017), is one such species. It is widely regarded as a nuisance in brackish water ponds, particularly in environments where natural predators and competitors are scarce (Zvonareva & Kantor, 2016).

To manage these pest populations, several chemical control methods have been introduced, including the use of triphenyltin (TPT) compounds, Aquatin, and Brestan. Although effective in reducing snail populations, TPT is highly toxic to non-target organisms such as fish, algae, bacteria, and even humans. Due to its detrimental environmental and health impacts, the Philippine Department of Agriculture banned the use of TPT in 1993 following reports of poisoning among rice farmers (Bagarinao & Lantin-Olaguer, 2000). Consequently, the search for sustainable, environmentally friendly, and cost-effective alternatives to chemical control remains a pressing concern in aquaculture management.

Despite their status as pests, mollusks like *Pirenella* sp. possess a favorable nutritional profile (Warsidah et al., 2024) that justifies their selection as a dietary supplement for farmed fish. Generally, gastropods are recognized as a dense source of high-quality animal protein, containing essential amino acids necessary for growth and tissue repair (Batista & Pires, 2022; Warsidah et al., 2024). Furthermore, they are rich in essential minerals, particularly calcium and phosphorus—which are vital for skeletal mineralization—as well as trace elements such as iron, magnesium, and zinc (Batista & Pires, 2022; Warsidah et al., 2024). By leveraging these nutritional benefits, these snails can be transitioned from a biological nuisance into a functional, low-cost feed ingredient.

The snubnose pompano (*Trachinotus blochii*), locally known as "apahan" or "dawis lawin," is a high-value marine fish recognized for its rapid growth, high-quality flesh, and strong market demand. This species has been reported in Indian coastal catches since 1956 and has become an important aquaculture species in several Asia-Pacific countries, including Taiwan and Indonesia (FAO, 2025). Juvenile pompano typically inhabits coastal sandy shores and shallow estuarine bays, while adults are found in offshore coral and rocky reef areas. They are diurnal feeders that consume mollusks, crustaceans, and other hard-shelled invertebrates, such as coquina clams and mole crabs (Froese & Pauly, 2025).

Given their natural feeding preference for mollusks, snubnose pompano may serve as potential biological control agents against pest gastropods like *Pirenella* sp. Utilizing these snails as a supplementary feed could offer dual benefits—reducing pest populations in ponds while providing a cost-effective source of natural feed. In intensive aquaculture systems, feed expenses account for nearly half of the total operational cost (SEAFDEC/AQD, 2024). Therefore, the use of locally available, underutilized, and nutritionally viable organisms such as *Pirenella* sp. may enhance the economic efficiency and sustainability of pompano culture operations.

This study explores the potential of *Pirenella* sp. as a supplemental feed for snubnose pompano and evaluates its capacity to contribute to the biological control of pest snails in brackish water ponds. If proven effective, this approach could significantly reduce the reliance on synthetic molluscicides and lower feed costs, thereby minimizing toxic chemical inputs in aquaculture environments. Moreover, the initiative aligns with several United Nations Sustainable Development Goals (SDGs): SDG 2 (Zero Hunger) by promoting sustainable and resilient food production systems; SDG 12 (Responsible Consumption and Production) by encouraging the use of locally available and underutilized resources; and SDG 14 (Life Below Water) by supporting sustainable aquaculture practices that protect aquatic biodiversity and reduce pressure on wild fish stocks. Overall, this research seeks to contribute to the advancement of environmentally sound, economically viable, and ecologically sustainable aquaculture practices in the

Philippines through the integration of natural pest control and resource-efficient feeding strategies.

MATERIAL AND METHODS

Study Area

The experiment was conducted at Sitio Calubuyan, Barangay Bantud Fabrica, Dumangas, Iloilo, Philippines, within the Dumangas Brackishwater Station (DBS) of SEAFDEC/AQD (Figure 1). The study utilized a 0.5–0.8 ha brackish water pond equipped with a double gate system (wood or concrete) and a separate supply and drainage canal. The pond bottom was leveled, with a gentle slope toward the gate to facilitate complete drainage.

Experimental Design and Setup

The study employed a completely randomized design (CRD) consisting of three treatments, each performed in triplicate (n=3). A total of nine black nylon hapa nets, each measuring 5 × 5 × 1.5 m, were suspended within a common earthen pond.

T. blochii (pompano) fingerlings, with an initial mean body weight (MBW) range of 120.90±.20 g to 133.43±3.2 g, were acclimated for two weeks prior to the commencement of the study. The fish were stocked at a density of 100 individuals per hapa (equivalent to 2.67 fish/m^3), totaling 300 fish per treatment and a collective experimental population of 900 individuals.

The use of triplicate hapa nets per treatment was selected to balance statistical power with the logistical requirement of maintaining identical water quality and minimizing environmental stressors across all units. This level of replication is consistent with established protocols for large-scale field trials in fish nutritional and performance studies (Kalidas et al., 2012; Babu et al., 2025), ensuring that inter-unit variability is accounted for while maintaining the feasibility of the trial within a common pond ecosystem.

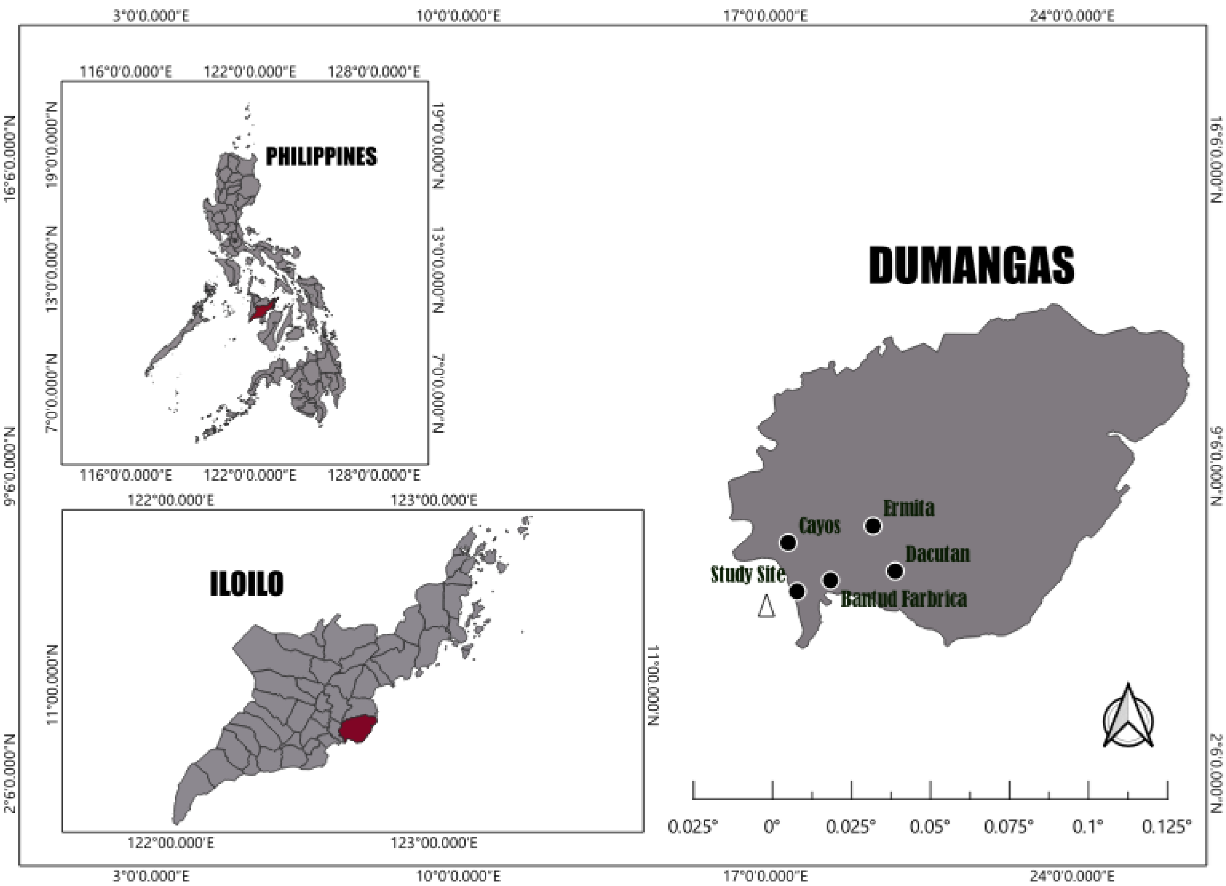

Figure 1. Experimental site located at the Dumangas Brackish Station of the SEAFDEC/AQD, Dumangas, Iloilo, Philippines

Pond Preparation

The pond was completely drained and sun-dried for 1-2 weeks until surface cracks appeared. The topsoil layer containing accumulated organic waste was removed, and the pond bottom was plowed to a depth of approximately 30 cm. Feeding areas, corners, and side ditches were tilled and dried to prevent anaerobic "black soil" formation. Agricultural lime was applied to neutralize soil pH. Prior to water filling, the inlet pipe was fitted with two layers of 100-μm fine mesh to prevent the entry of unwanted organisms.

Water Culture and Management

Water was sourced by gravity flow or pumping from a reservoir. The pond was fertilized one week prior to stocking with organic (10-30 kg/ha) and inorganic (1-3 kg/ha) fertilizers to promote plankton bloom, essential for early pompano growth. Water depth was maintained at 1 m throughout the culture period. A weekly 10% water exchange was implemented during the first three months, increasing to 20% thereafter. Agri-lime was applied regularly to maintain pH between 7.5 and 8.5. Water quality parameters recorded during the experiment were given in Table 1.

Table 1. Water quality parameters maintained during the experiment

Parameter	Range
Salinity (‰)	25-33
Temperature (°C)	26-32
Dissolved oxygen (ppm)	4-8
pH	6.5-8.5
Transparency (cm)	35-45
Water color	Light green to brownish-green

Source of Fingerlings and Feed Materials

Snubnose pompano juveniles (average body weight ranging from 120.9 ± 0.20 g to 133.43 ± 3.20 g and total length from 19.14 ± 0.06 cm to 19.46 ± 0.19 cm) were obtained from the SEAFDEC/AQD Dumangas Brackishwater Station. *Pirenella* sp. snails were collected from nearby snail-infested ponds and identified taxonomically before use. Only young, active snails were selected for feeding.

Stocking and Feeding Management

Snubnose pompano juveniles were stocked at a density of 100 individuals per hapa net for each treatment, with a total of 900 fish used in the experiment. The fish were fed three times daily throughout the culture period. Treatment I fish were fed exclusively with commercial pompano feed, while Treatment II received a mixed diet consisting of 50% commercial feed and 50% snail meat derived from *Pirenella* sp. Treatment III fish were provided with whole *Pirenella* sp. snails only, which were placed in feeding trays inside the hapa nets. To ensure experimental control, each hapa was covered with a fine-mesh net to prevent snail escape and to protect the fish from potential predators.

Proximate Composition of the Commercial Feeds and Snails

The proximate compositions (moisture content, crude protein, crude lipid, ash, and crude fiber) of the whole *Pirenella* sp. (shell and soft tissue) and commercial feeds were determined following AOAC (2016) standard procedures. Moisture content was determined using the gravimetric method (oven drying at 105°C). Crude protein was analyzed using the Kjeldahl method, with nitrogen converted to protein using a factor of 6.25. Crude lipid was investigated using Soxhlet extraction with a non-polar solvent. Ash content was determined via the dry ashing method (muffle furnace incineration at 550-600°C). Lastly, crude fiber was determined through sequential acid-alkali digestion of the defatted sample.

The proximate composition of the commercial feeds used in the experiment is presented in Table 2. The crude protein content of the feeds ranged from 44% to 48%, with PO1 and PO2 containing the highest levels (48%), followed by PN2F (46%) and PN3F-PN4F (44%). All feeds had a uniform moisture content of 12% and crude fiber content of 4%. The crude fat content varied between 7% and 14%, with PN2F showing the highest value (14%), while the ash content ranged from 15% to 16%.

Meanwhile, the proximate composition of *Pirenella* sp. snails (Table 3) revealed that the snail meat contained 4.81% crude protein, 43.11% moisture, 0.26% crude fat, 4.35% crude fiber, and 89.83% ash. These results indicate that *Pirenella* sp. has relatively low protein and lipid contents compared to commercial feeds but is rich in mineral components, as reflected by its high ash content.

Table 2. Proximate composition of commercial feeds

Nutrient	PN3F (1-30 days)	PN4F (31-60 days)
Crude protein (%)	44	44
Moisture (%)	12	12
Crude fat (%)	12	12
Crude fiber (%)	4	4
Ash (%)	15	15

Table 3. Proximate composition of *Pirenella* sp. snails

Nutrient	Value (%)
Protein	4.81
Moisture	43.11
Crude fat	0.26
Crude fiber	4.35
Ash	89.83

Sampling and Monitoring

Growth performance and survival were assessed every 15 days (Days 0, 15, 30, 45, and 60). At the start of the trial, each replicate was stocked with 100 fish. During each 15-day sampling event, all fish remaining in the cage were measured for total length and body weight to calculate absolute body weight (ABW), specific growth rate (SGR), and weight gain (WG). Following these measurements, 10 fish per replicate were randomly selected and removed for gut content analysis (independent study; results not included here). Consequently, the number of fish measured per replicate decreased by 10 at each subsequent sampling interval (e.g., 100 fish at Day 0, 90 fish at Day 15, etc.)

In Treatments II and III, snail consumption was monitored by counting and weighing remaining snails and recording cracked shells as indicators of feeding activity.

Water quality parameters (pH, salinity, temperature, dissolved oxygen, and turbidity) were measured twice daily (morning and afternoon).

Data Collection and Calculations

To evaluate the growth performance and feed utilization of the fish, the following parameters were calculated according to standard formulas given below in Equations 1-5:

$$ABW = \frac{Total\ weight\ of\ fish\ samples}{Number\ of\ fish\ sampled} \quad (1)$$

$$WG = Final\ weight - Initial\ weight \quad (2)$$

$$SGR = \frac{\ln(Final\ weight) - \ln(Initial\ weight)}{Number\ of\ days} \times 100 \quad (3)$$

$$Survival\ Rate(\%) = \frac{Final\ number\ of\ live\ fish}{Total\ number\ of\ fish\ stocked} \times 100 \quad (4)$$

$$FCR = \frac{Total\ feed\ consumed\ (dry\ weight)}{Wet\ weight\ gain\ of\ fish} \quad (5)$$

To ensure a standardized comparison between the dry commercial diet and the live snail diet, feed conversion ratio (FCR) was calculated on a dry matter basis. Live snails (including shells) were oven-dried at 60°C to determine a dry matter content of 43.11 %. Total feed intake for Treatments II and III was then adjusted to reflect dry matter intake before calculating FCR.

Data Analysis

Growth and survival data were subjected to one-way analysis of variance (ANOVA) at a 5% significance level to determine differences among treatments. Where significant differences were observed, least significant difference (LSD) and Tukey’s HSD post-hoc tests were applied for mean comparison.

RESULTS AND DISCUSSION

Growth Performance

The present study evaluated the effects of dietary composition on the growth performance and survival of snubnose pompano (*T. blochii*) over a 60-day culture period. Three dietary treatments were tested: Treatment I: 100% commercial feed, Treatment II: 50% commercial feed + 50% snails, and Treatment III: 100% snail-based feed.

Table 4 presents the mean initial average body weight (ABW) and total length of snubnose pompano at stocking. Initial sizes were comparable across treatments, with ABW ranging from 120.9 ± 0.20 g to 133.43 ± 3.20 g and total length from 19.14 ± 0.06 cm to 19.46 ± 0.19 cm. There were no significant differences ($p > 0.05$) in total length, indicating uniform initial conditions among treatments.

Table 4. Mean initial average body weight (ABW) and length of snubnose pompano *Trachinotus blochii*

Growth Parameters	Treatment I	Treatment II	Treatment III
ABW	125.86±2.2	133.43±3.2	120.90±.20
Total length	19.22±.20	19.14±.06	19.46±.19

Table 5. Growth performance of snubnose pompano (*Trachinotus blochii*) every 15 days, fed with 100% feeds, 50% feeds and 50% snails, and 100% snails for 60 days of culture

Parameters	Day	Treatment I	Treatment II	Treatment III
Average body weight	15	160.53±1.6^{a}	163.39±6.81^{a}	127.30±.30^{b}
Weight gain (g)		34.66±2.1^{a}	30.09±9.4^{a}	6.43±.49 ^{b}
Specific growth rate		2.31±.14^{a}	2.00±.62^{a}	0.42±0.33^{b}
Total length		20.11±1.40^{a}	20.78±.22^{a}	19.78±.17^{a}
Survival rate (%)		100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Average body weight	30	175.66±1.4^{a}	177.51±6.7^{a}	133.11±1.52^{b}
Weight gain (g)		15.1±3.02^{a}	14.06±1.27^{a}	5.83±1.70^{b}
Specific growth rate		1.00±.20^{a}	0.93±.08^{a}	0.38±.11^{b}
Total length		21.33±.19^{a}	22.09±.14^{a}	20.32±.06^{b}
Survival rate (%)		100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Average body weight	45	193.76±1.2^{a}	194.5±3.5^{a}	134.92±1.4^{b}
Weight gain (g)		18.06±.48^{a}	17.09±5.5^{a}	1.81±.08^{b}
Specific growth rate		1.20±.03^{a}	1.13±.37^{a}	0.73±.62^{b}
Total length		22.54±.09^{a}	22.97±.11^{a}	20.94±.12^{b}
Survival rate (%)		100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Average body weight	60	211.43±3.30^{a}	215.88±4.34^{a}	138.3±1.06^{b}
Weight gain (g)		21.00±2.70^{a}	23.21±3.65^{a}	3.46±.39^{b}
Specific growth rate		1.40±.18^{a}	1.46±.27^{a}	0.22±.02^{b}
Total length		23.99±.05^{a}	24.25±.10^{a}	21.76±.24^{b}
Survival rate (%)		100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Note: *Values are expressed as mean ± standard error (n = 3). Means in the same row with different superscript letters are significantly different ($p < 0.05$) based on one-way ANOVA.

Growth performance results are summarized in Table 5. During the first 15 days, snubnose pompano fed with 100% commercial feed (Treatment I) exhibited the highest mean weight gain (34.66 ± 2.1 g), followed by the 50:50 feed-snail group (30.09 ± 9.4 g), while the snail-fed group (Treatment III) showed minimal gain (6.43 ± 0.49 g). This trend continued through Day 30 and Day 45, where Treatments I and II maintained significantly higher growth rates than Treatment III ($p < 0.05$). By the end of the 60-day culture, Treatment II (50% commercial feed + 50% snails) achieved the highest mean weight gain (23.21 ± 3.65 g), slightly higher but statistically comparable to Treatment I (21.00 ± 2.70 g). Both treatments showed markedly better performance than the snail-only diet (3.46 ± 0.39 g). The specific growth rate (SGR) after 60 days were 1.40 ± 0.18 % day^{-1} for Treatment I, 1.46 ± 0.27% day^{-1} for Treatment II, and 0.22 ± 0.02% day^{-1} for Treatment III.

These results clearly indicate that snubnose pompano fed with 100% commercial feed or a 50:50 mixture of feed and snails exhibited superior growth performance compared with those fed only with snails. The comparable growth between Treatments I and II suggests that partial replacement of commercial feed with snail meat did not compromise nutrient intake or growth potential.

Formulated feeds are specifically designed to provide balanced levels of protein, lipid, and essential micronutrients required by snubnose pompano (Viet et al., 2022). The comparable performance of Treatment II supports the possibility that snail meat can supply a portion of the animal protein requirements, potentially improving feed palatability and stimulating feeding behavior. Similar findings have been reported in *T. ovatus*, where partial replacement of fishmeal with alternative protein sources did not significantly affect growth (Ma et al., 2020).

The markedly low growth observed in Treatment III aligns with findings in other carnivorous species such as cobia (*Rachycentron canadum*), where natural mollusk diets alone resulted in poor performance due to amino acid imbalance and low energy density (Oliva-Teles et al., 2006). The low SGR in this treatment (0.29% day^{-1}) likely reflects inadequate digestible protein and lipid content in snails (Milinsk et al., 2006), underscoring the importance of balanced formulated diets in intensive culture systems.

Survival Rate

Survival rates remained at 100% across all treatments throughout the 60-day period, with no significant differences among groups ($p > 0.05$). This indicates that all diets supported basic physiological maintenance and that the culture environment was conducive to fish health.

High survival suggests that experimental conditions such as water quality, stocking density, and feeding protocols were optimal. These results are consistent with earlier studies on *T. blochii*, which reported survival rates exceeding 95% under good pond management (Juniyanto et al., 2008; Jayakumar et al., 2014).

The ability of snubnose pompano to thrive under varying salinities (Young et al., 2021) and its resilience against common marine pathogens (Lan et al., 2022) may have also contributed to these outcomes. Maintaining optimal temperature, dissolved oxygen, and pH likely minimized stress, preventing immune suppression and mortality (Tacon & Metian, 2015).

While dietary composition had a marked effect on growth, it did not influence survival. This observation agrees with Glencross et al. (2016), who reported that juvenile snubnose pompano can maintain high survival even on suboptimal diets, though growth may be impaired. Thus, survival reflects the adequacy of environmental conditions, whereas growth performance more directly indicates nutritional sufficiency.

Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) values after 60 days are summarized in Table 6. Treatment II (50% commercial feed + 50% snails) achieved the lowest FCR values (0.34 ± 0.01), indicating the most efficient feed utilization. Treatment I (100% commercial feed) recorded slightly higher FCRs (0.47 ± 0.01), while Treatment III (100% snails) showed the highest FCR (1.13 ± 0.12), reflecting poor feed efficiency.

Table 6. Average feed conversion ratio (FCR) of *Trachinotus blochii* fed with different dietary treatments for 60 days

Treatment	**FCR**
Treatment I	0.47 ± 0.02[b]
Treatment II	0.34 ± 0.01[c]
Treatment III	1.13 ± 0.12[a]

Note: Treatment I (100% commercial feed), Treatment II (50% commercial feed + 50% snails), Treatment III (100% snails). Values are mean ± standard error (n=3). Means in the same column with different superscript letters are significantly different ($p < 0.05$) based on one-way ANOVA followed by Tukey's HSD test.

The superior FCR in Treatment II likely resulted from the complementary nutritional properties of the two feed components. Commercial diets meet the full nutritional requirements of snubnose pompano (Glencross et al., 2016; Lan et al., 2022), while snail meat provides fresh animal protein that enhances palatability and feed intake. The synergy between formulated feed and natural feed may have improved nutrient digestibility and assimilation, similar to results observed in other marine fish supplemented with natural ingredients (Yones & Metwalli, 2015).

The moderately higher FCR in Treatment I may be due to the absence of natural feeding stimuli found in mixed diets, which can enhance enzyme activity and digestive efficiency (De Silva & Anderson, 1995). Conversely, the high FCR of the snail-only group (Treatment III) can be attributed to the limited nutrient content of snails, particularly deficiencies in essential amino acids and fatty acids (Young et al., 2021). Variable nutrient composition may also have contributed to inconsistent intake and growth (Shi et al., 2023).

From an economic perspective, Treatment III had the lowest feed cost due to the availability of snails as a natural resource in brackish water ponds. However, its poor growth performance limits its suitability for commercial use. Treatment II offers a more balanced approach, reducing commercial feed costs by 50% while maintaining efficient feed utilization and good growth. Similar results were reported by Pham et al. (2021), where supplementing formulated feeds with low-cost natural ingredients reduced production expenses without compromising performance.

CONCLUSION

This study demonstrated that the partial replacement of commercial feed with *Pirenella* sp. in the diet of snubnose pompano (*T. blochii*) can sustain optimal growth and feed utilization while maintaining 100% survival. Fish fed with a 50:50 ratio of commercial feed and snails exhibited comparable growth performance and superior feed conversion efficiency to those fed solely with commercial diets, indicating that *Pirenella* sp. can serve as an effective, sustainable, and low-cost supplemental feed. In contrast, exclusive feeding with snails resulted in poor growth, underscoring the need for balanced nutrient formulation. The integration of *Pirenella* sp. into snubnose pompano culture systems not only reduces feed costs but also provides a natural means of controlling pest snail populations in brackish water ponds, contributing to more eco-friendly and cost-efficient aquaculture practices. Further studies should focus on optimizing the inclusion levels of *Pirenella* sp. in formulated diets, evaluating its nutritional digestibility, and assessing long-term effects on fish health and product quality under different culture conditions.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the following individuals for their invaluable assistance and technical support during the conduct of this study: Jesus G. Apelacio, Jefran T. Arellado, May Vincent A. Arellano, Julie B. Biogos, Roselie S. Cabatia, John Andrew C. Cercado, Joshua V. Dela Peña, Ralen A. Depamaylo, Mary Jane D. Embalsado, Roderix E. Escosa, Brench T. Genzola, Johnbert L. Godio, Efren J. Gonzales Jr., Felix J. Javero, Dominador J. Leabres, Dominic J. Leabres, Melben M. Magpili, Lister G. Salvaria, Russel D. Somblingo, and Ronnie H. Teñefrancia.

Compliance with Ethical Standards

Authors' Contributions

EFS: Conceptualization, Supervision, Writing – original draft, Funding acquisition, Formal Analysis.

DDB: Investigation, Methodology.

REPM: Investigation, Methodology.

ABC: Investigation, Methodology.

EFS: Conceptualization, Supervision, Writing – review and editing.

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

Approval was obtained from the ethics committee of Iloilo State University of Fisheries Science and Technology. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Funding

This study was financially supported by Southeast Asian Fisheries and Development Center-Aquaculture Department (SEAFDEC/AQD) with grant number: 5310-T-TV-F0224D.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

The authors confirm that no generative AI was used in writing this manuscript or creating images, tables, or graphics.

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Acta Nat. Sci. (2026) 7(1): 54-64
DOI: 10.61326/actanatsci.v7i1.465
e-ISSN: 2718-0638

Original Research Article
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ACTA NATURA ET SCIENTIA PRENSIP

Interannual Variability of Reservoir Occupancy: A Multi-Reservoir Assessment from Çanakkale, Türkiye

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Please cite this paper as follows:
Yıldırım, P. (2026). Interannual Variability of Reservoir Occupancy: A Multi-Reservoir Assessment from Çanakkale, Türkiye. *Acta Natura et Scientia*, *7*(1), 54-64. https://doi.org/10.61326/actanatsci.v7i1.465

ARTICLE INFO

Article History
Received: 13.02.2026
Revised: 11.05.2026
Accepted: 13.05.2026
Available online: 12.06.2026

Keywords:
Reservoir occupancy
Interannual variability
Trend analysis
Similarity analysis
Water resource management

ABSTRACT

Monitoring reservoir occupancy rates is increasingly important under rising water demand and growing hydrological variability. This study evaluates temporal variability in occupancy rates of seven reservoirs (Atikhisar, Ayvacık, Bakacak, Gökçeada, Taşoluk, Tayfur, and Umurbey) in Çanakkale Province (Türkiye) over the period 2013–2023. Bray–Curtis similarity and hierarchical cluster analyses were applied to assess interannual and inter-reservoir patterns, while Mann Kendall trend analysis, Sen's slope estimator, and Spearman's rank correlation were also applied to evaluate temporal trends and precipitation occupancy relationships. The results revealed interannual variability, with occupancy rates ranging from 2.7% to 79.2%. Gökçeada, Atikhisar, and Umurbey exhibited relatively similar occupancy dynamics, whereas Bakacak and Taşoluk displayed more distinct patterns. Trend analysis indicated that no statistically significant trends were observed for most reservoirs ($p > 0.05$), whereas a statistically significant decreasing trend was detected only for Umurbey Reservoir ($Z = -2.14$, $p = 0.033$; Sen's slope = -2.45). Correlation analysis did not indicate a statistically consistent relationship between annual precipitation and reservoir occupancy ($p > 0.05$), despite moderate correlation coefficients in some reservoirs. These results indicate that annual precipitation alone does not adequately explain interannual variations in reservoir occupancy. Overall, reservoir occupancy appears to reflect the combined influence of hydroclimatic variability and other unquantified factors rather than a single controlling variable. The results provide a quantitative and comparative assessment of reservoir occupancy dynamics and may serve as a reference for future water management and research efforts in the region.

INTRODUCTION

Water continuously circulates between the Earth's surface and the atmosphere through the hydrological cycle. Although approximately three-quarters of the Earth's surface is covered by water, about 97–98% of it is stored in oceans and inland seas. Due to its salinity, this water is not suitable for drinking, irrigation, or industrial use (Abdelali, 2019). Freshwater, which constitutes only a small fraction of total water resources, is an indispensable component for sustaining biological life and human societies. While a significant portion of freshwater is stored in glaciers and groundwater, the amount of accessible surface water is limited. This limited availability makes freshwater a vital and strategic resource (Gündoğdu, 2025). In addition, hydrological events such as irregular precipitation, floods, and droughts resulting from global warming threaten the availability of freshwater. The conservation and sustainable management of water resources require planned and efficient use of existing supplies, particularly in regions where water demand is steadily increasing. Therefore, monitoring water occupancy rates, especially in dam reservoirs is crucial for ensuring reliable and sustainable freshwater resources.

Dams are basic engineering structures that are built with significant investments and are expected to serve for a very long time (Aşık, 2016). Dams are structures that serve a wide variety of purposes, including domestic and industrial water supply, agricultural irrigation, flood control, hydroelectric energy production and ecosystem regulation (Taş-Divrik, 2022). Regular monitoring of dam water levels is essential for developing water management policies and establishing early-warning mechanisms for potential drought scenarios. Recent searches have increasingly focused on temporal variability in reservoir occupancy rates and their relationship with precipitation, as well as comparative analyses among multiple reservoirs to better understand regional water dynamics (Hou et al., 2022; Steyaert et al., 2022; Demirbaş & Özbaş, 2024; Cui & Liu, 2025; Akiner, 2025). However, despite the presence of several important reservoirs, studies focusing specifically on Çanakkale Province remain limited in the literature.

Located in Çanakkale Province, Atikhisar reservoir, the primary drinking water source for the city center, holds strategic importance. Ayvacık, Bakacak, Tayfur and Umurbey reservoirs provide multi-purpose water supply, particularly for agricultural irrigation. Gökçeada reservoir serves island settlements and their surrounding rural areas, while Taşoluk reservoir supports increased irrigation demand during the summer months.

This study addresses the following research questions:

(i) Are there statistically significant temporal trends in reservoir occupancy rates?

(ii) Does annual precipitation explain interannual variability in occupancy rates?

(iii) Do reservoirs exhibit similar or distinct occupancy patterns? From this perspective, this study analyzed the occupancy rates of the Atikhisar, Ayvacık, Bakacak, Gökçeada, Taşoluk, Tayfur, and Umurbey dam reservoirs in Çanakkale Province between 2013 and 2023, using data published by the General Directorate of State Hydraulic Works. This study contributes to the literature by integrating trend analysis (Mann–Kendall and Sen's slope), correlation analysis (Spearman's rank correlation), and similarity based clustering (Bray–Curtis) to evaluate reservoir occupancy dynamics. From an applied perspective, the findings provide information for regional water management.

MATERIAL AND METHODS

Study Area and Data Source

This study analyzed annual reservoir occupancy rate data for seven reservoirs in Çanakkale Province, Türkiye. The spatial distribution of the investigated reservoirs is shown in Figure 1. Occupancy rates (%) for Atikhisar, Ayvacık, Bakacak, Gökçeada, Taşoluk, Tayfur, and Umurbey reservoirs were obtained from the General Directorate of State Hydraulic Works of the Republic of Türkiye. The dataset covers the period 2013–2023, which was specifically selected because occupancy records prior to 2013 contained gaps for some reservoirs, preventing a consistent inter-reservoir comparison. The occupancy rates represent annual values (%) as officially reported for each year, providing a complete dataset for interannual analysis. These values represent annual occupancy percentages as officially reported by the State Hydraulic Works (Anonymous, 2025a) and are not derived from monthly or daily averaging within this study. Annual precipitation data for the study region were obtained from the provincial briefing reports of the Provincial Directorate of Agriculture and Forestry, which compile measurements from the Turkish State Meteorological Service (Anonymous, 2025b). These datasets were used to examine temporal variability in reservoir occupancy rates and to investigate potential relationships with precipitation.

Figure 1. Spatial distribution of the investigated reservoirs in Çanakkale Province, Türkiye

Structural Characteristics of the Reservoirs

The structural and operational characteristics of the investigated reservoirs are summarized in Table 1. These characteristics include construction period, body fill type, body volume, reservoir volume, reservoir area, and primary purposes such as irrigation, potable water supply, and flood control. Detailed technical specifications for each reservoir are provided in Table 1.

Data Processing and Statistical Analysis

All percentage-based reservoir occupancy rate data were first examined to ensure completeness and consistency. Microsoft Excel was used for data organization, preprocessing, and calculation of descriptive statistics, including minimum, maximum, mean values, standard deviation (SD), and coefficient of variation (CV) to characterize variability in reservoir occupancy rates.

Reservoir occupancy rates and annual precipitation data were evaluated on a yearly basis throughout the study period (2013–2023) to assess interannual variability.

Monotonic trends in annual reservoir occupancy rates were analyzed for each reservoir using the non-parametric Mann–Kendall trend test implemented in PAST (PAleontological STatistics) software, version 4.17 (Hammer et al., 2001). The magnitude of trends was further estimated using Sen's slope estimator (Sen, 1968). Sen's slope values were calculated in Microsoft Excel using the median of all pairwise slopes between data points, expressed as (Eq. 1):

$$Q = \text{Median}\left(\frac{x_i - x_j}{j-i}\right),\ j > i \qquad (1)$$

where x_i and x_j represent reservoir occupancy rates at times i and j, respectively.

The relationship between annual precipitation and reservoir occupancy rates was evaluated using Spearman's rank correlation analysis. Annual precipitation data represent spatially aggregated values at the provincial scale and were used as a regional indicator of climatic conditions influencing all reservoirs. This nonparametric method was selected due to the non-normal distribution and limited length of the dataset.

Similarity analysis among reservoirs was conducted to identify comparable occupancy patterns. Annual occupancy rates (2013–2023) were used to construct a Bray–Curtis similarity matrix based on temporal occupancy profiles. Hierarchical cluster analysis was then applied using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

In addition, interannual similarity was assessed by treating each year as a sample and reservoirs as variables. All correlation, similarity and clustering analyses were performed using PAST 4.17.

Table 1. Structural characteristics of the investigated reservoirs in Çanakkale Province, Türkiye

Reservoir	Purpose	Built Date	Body Fill Type	Body Volume (m^3)	Height (m)	Stream (Outgoing)	Reservoir Volume (hm^3)	Reservoir Area (km^2)	Irrigation Area (ha)
Atikhisar	Irrigation, Flood Control, Potable water supply	1971-1975	Soil	1,990,000	43	Sarıçay Creek	40	3.30	5200
Ayvacık	Irrigation	1997-2007	Soil	1,200,000	53	Tuzla Creek	30	3.42	3900
Bakacak	Irrigation	1991-1999	Rock	1,750,000	65	Koca Creek	139	7.74	9100
Gökçeada	Potable water supply, Irrigation	1977-1983	Soil	561,000	51	Büyükdere Creek	14	1.35	700
Taşoluk	Irrigation	1995-2001	Rock	1,700,000	75	Çınarcık Creek	88	3.10	7924
Tayfur	Potable water supply	1980-1985	Rock, Soil	298,000	39	Tayfur Creek	4.36	0.47	-
Umurbey	Irrigation	1995-2003	Soil	2,400,000	81	Umurbey Creek	24.56	1.70	2445

Table 2. Reservoir occupancy rates of Atikhisar, Ayvacık, Bakacak, Gökçeada, Taşoluk, Tayfur, and Umurbey and annual precipitation amounts (mm) for Çanakkale province between 2013-2023

Reservoir	Reservoir Occupancy Rates (%)														
	2013	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**	**Max**	**Min**	**Mean**	**SD**	**CV (%)**
Atikhisar	52.7	47.8	55.7	33.1	30.9	61.7	57.5	25.5	79.2	49.1	33.4	79.2	25.5	48.56	16.02	33.47
Ayvacık	60.4	60.9	42	34.6	39.6	36.8	48	4.5	68.3	43.9	35.7	68.3	4.5	42.12	17.16	39.77
Bakacak	33.6	22.4	27.9	2.7	5.6	14	44.3	14.1	11.5	8.7	16.5	44.3	2.7	19.10	12.65	69.15
Gökçeada	65.7	69.1	63.4	43.5	30.3	63.6	68.2	48.8	70.1	56.1	36.7	70.1	30.3	55.07	14.02	25.06
Taşoluk	24.3	24.4	40.1	24.5	23.9	11.4	30.2	27.4	27	18.4	7.7	40.1	7.7	23.62	8.79	37.29
Tayfur	50.9	52.2	44.7	26.1	28.8	63.1	52.6	32.3	35	30.7	18.8	63.1	18.8	39.78	13.85	35.01
Umurbey	43.1	62.2	57.9	43.1	48.6	60.7	49.1	40.7	55.7	35.8	21	62.2	21	46.24	12.21	25.94
Annual precipitation (mm)	650.2	753.3	650.3	570.9	648.6	660.1	581.7	453	749.6	480.7	711.4	453	753.3	624.77	98.80	15.73

RESULTS

In this study, reservoir occupancy rates of Atikhisar, Ayvacık, Bakacak, Gökçeada, Taşoluk, Tayfur, and Umurbey reservoirs in Çanakkale Province were analyzed for the period 2013–2023 using the statistical methods. Reservoir occupancy rates varied between 2.7% and 79.2% during the study period (Table 2). Mean values presented in Table 2 represent the arithmetic average of annual occupancy rates for the period 2013–2023. Furthermore, the relationship between reservoir occupancy rates and average annual total precipitation is shown in Figure 2.

All reservoirs exhibited negative Sen's slope values, indicating an overall decreasing tendency in occupancy rates, although these trends were not statistically significant except for Umurbey ($Z = -2.14$, $p = 0.033$; Sen's slope = -2.45) (Mann-Kendall test, $p > 0.05$) (Table 3).

Spearman's rank correlation analysis indicated that correlation coefficients between annual precipitation and reservoir occupancy rates ranged from −0.12 to 0.57 (Table 4). Although moderate positive associations were observed in some reservoirs (e.g., Umurbey, $r_s = 0.57$; Ayvacık, $r_s = 0.52$), none of the correlations were statistically significant ($p>0.05$).

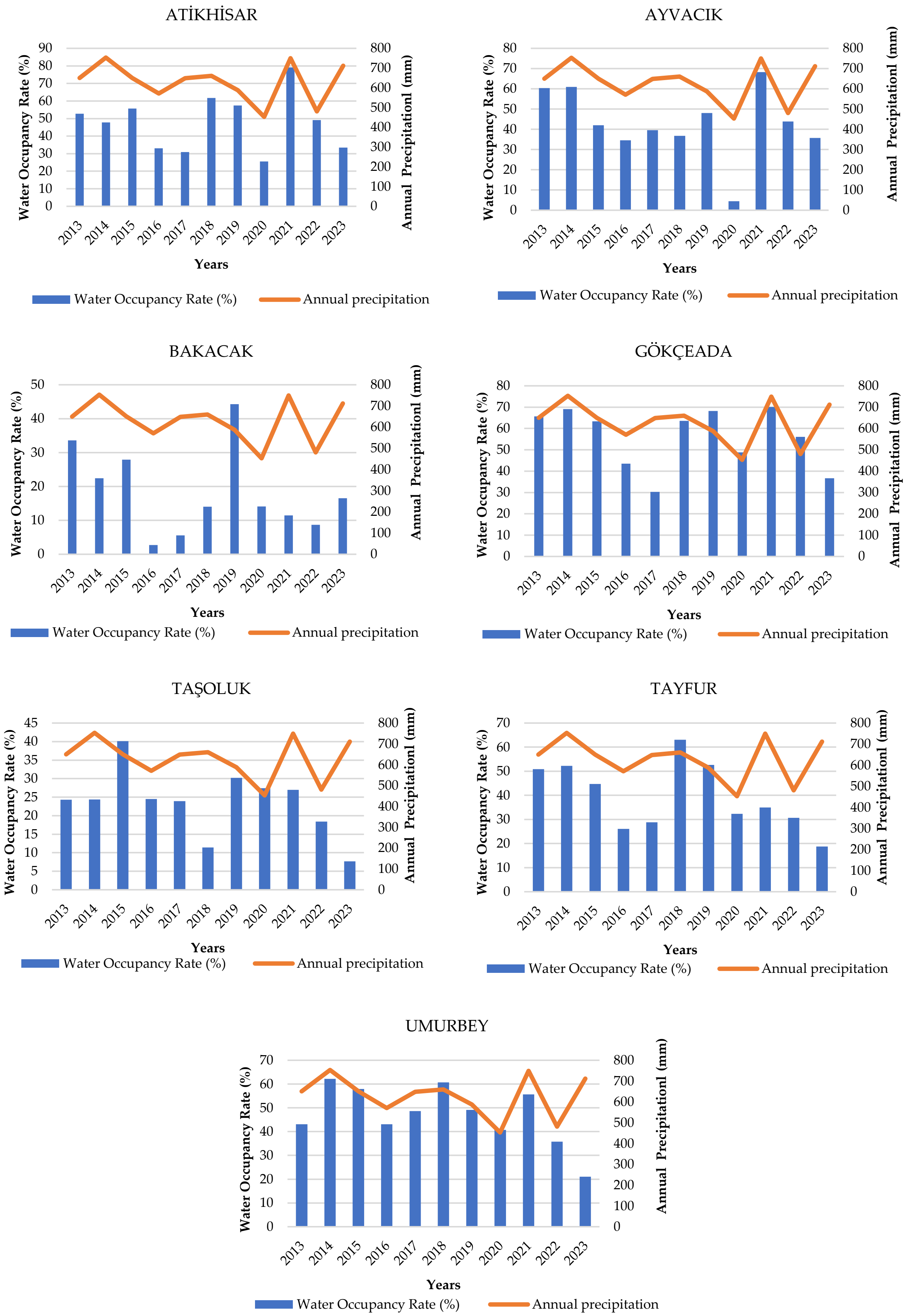

Figure 2. Total precipitation and occupancy rates of Atikhisar, Ayvacık, Bakacak, Gökçeada, Taşoluk, Tayfur, and Umurbey reservoirs between 2013 and 2023

Table 3. Mann–Kendall trend test and Sen's slope results for reservoir occupancy rates (2013-2023)

Reservoir	n	S	Z	p value	Sen's slope	Trend
Atikhisar	11	-5	-0.27	0.783	-0.40	No significant trend
Ayvacık	11	-17	-1.10	0.271	-1.73	No significant trend
Bakacak	11	-7	-0.41	0.680	-1.43	No significant trend
Gökçeada	11	-17	-1.10	0.271	-1.07	No significant trend
Taşoluk	11	-23	-1.51	0.130	-0.75	No significant trend
Tayfur	11	-27	-1.79	0.074	-2.48	No significant trend
Umurbey	11	-32	-2.14	**0.033**	-2.45	Decreasing trend

Table 4. Spearman's rank correlation coefficients (r_s) and associated p-values between annual precipitation and reservoir occupancy rates (2013–2023)

Reservoir	r_s	p value
Atikhisar	0.4636	0.1509
Ayvacık	0.51818	0.10249
Bakacak	0.24545	0.46692
Gökçeada	0.47273	0.142
Taşoluk	-0.12325	0.71807
Tayfur	0.28182	0.40114
Umurbey	0.56948	0.067443

According to the similarity analyses results, the years 2013 and 2014 exhibited the highest degree of similarity, with a similarity coefficient of 93.95%. These two years form a distinct cluster, indicating comparable reservoir occupancy conditions. In addition, 2019 was included within this cluster, showing a high similarity value of 91.84% (Figure 3; Table 5).

In contrast, the lowest similarity was observed between the years 2021 and 2023, with a similarity coefficient of 63.80%, indicating pronounced interannual differences in reservoir occupancy rates. The dendrogram further reveals a gradual divergence among the years, reflecting increasing variability in reservoir water levels over time. These patterns are consistent with the variability observed in Figure 2, where reservoir occupancy rates fluctuate across years.

Cluster analysis revealed a main group consisting of Gökçeada, Atikhisar, Ayvacık, Umurbey, and Tayfur reservoirs, which exhibited relatively high similarity coefficients. In contrast, Bakacak reservoir displayed lower similarity values with the other reservoirs, particularly with Gökçeada (0.49), indicating more distinct occupancy dynamics. Taşoluk reservoir also showed relatively lower similarity values compared with the main group. Similarity coefficients among the seven reservoirs are presented in Figure 4 and Table 6.

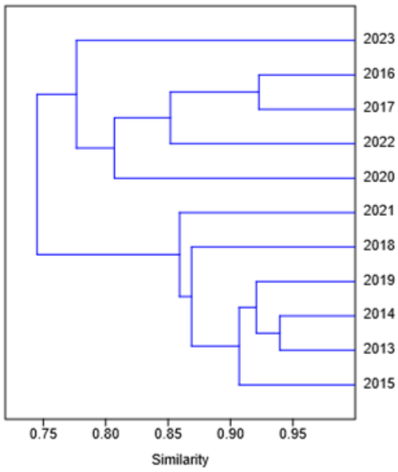

Figure 3. Dendrogram of reservoir occupancy rates for the period 2013–2023

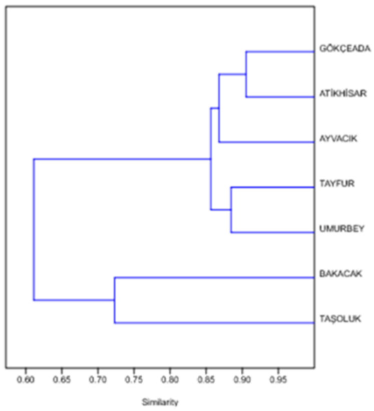

Figure 4. Dendrogram of reservoirs based on similarity matrix

Table 5. Similarity matrix of reservoir occupancy rates for the period 2013–2023

Year	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
2013		93.9525	90.006	77.0574	75.1114	84.891	93.5351	72.5954	86.4059	84.6529	67.8521
2014			90.2341	75.9239	75.9832	88.1132	90.6082	71.501	88.7722	82.9981	66.7453
2015				76.9887	77.0115	88.3048	91.8427	73.6381	85.5711	83.844	67.7169
2016					92.2706	74.9663	74.4753	83.562	74.8918	86.2536	81.929
2017						74.1426	74.4978	79.4514	74.9143	84.103	79.4702
2018							86.2069	70.2338	83.7867	82.5271	69.549
2019								71.1708	85.0581	81.9102	65.3454
2020									70.4684	79.0826	70.6692
2021										82.341	63.8018
2022											78.5455
2023											

Table 6. Similarity matrix among reservoirs

Reservoir	Atikhisar	Ayvacık	Bakacak	Gökçeada	Taşoluk	Tayfur	Umurbey
Atikhisar		0.88165385	0.55309795	0.90517468	0.65504517	0.87876897	0.87774055
Ayvacık			0.56715976	0.8537883	0.64414169	0.82162875	0.85029216
Bakacak				0.49289912	0.72297004	0.6325216	0.55978865
Gökçeada					0.59282122	0.82840011	0.8815952
Taşoluk						0.74672426	0.66726711
Tayfur							0.88448222
Umurbey							

DISCUSSION

Maintaining adequate water occupancy in surface reservoirs is essential, as water levels are generally influenced by seasonal hydroclimatic factors such as precipitation and snowmelt (Demirbaş & Özbaş, 2024). Previous studies have reported notable interannual variability in reservoir occupancy, particularly during drought periods. Furthermore, long term drought predictions and decreasing precipitation forecasts further complicate water management in dams (Ayyıldız & Erdoğan, 2022). For example, Dirican (2021) reported considerable fluctuations in Çermikler Dam, the potential roles of hydroclimatic variability and water use pressures in shaping reservoir dynamics (Dirican, 2023a, 2023b, 2024, 2025). Similar variability has also been reported for reservoirs supplying metropolitan areas such as Istanbul. Analyses of Istanbul's dams highlight that climate change is increasing volatility in water levels (Nalici & Akbaş, 2022; Akiner, 2025). Considering projections of increasing drought frequency and altered precipitation regimes on water resources, such variability may become more pronounced in the future (Türkeş, 1998; Taş-Divrik, 2022).

In the present study, the occupancy rates of seven reservoirs in Çanakkale Province showed marked interannual variability between 2013 and 2023 (Table 2, Figure 2). Reservoir occupancy values ranged from 2.7% to 79.2%, indicating substantial fluctuations across years (Table 2). Spearman's rank correlation analysis indicated that, although weak to moderate positive correlations were observed between annual precipitation and reservoir occupancy, these relationships were not statistically significant ($p > 0.05$) (Table 4). This suggests that annual total precipitation alone may not fully account for variations in reservoir occupancy during the study period. Consistent with this finding, the results of the Mann–Kendall trend test and Sen's slope estimator indicated that no statistically significant trends were observed in the majority of the reservoirs during the period 2013–2023 (Table 3). The calculated Z values were generally low and the corresponding p-values remained above the 0.05 significance level. However, all reservoirs exhibited negative Sen's slope values, indicating an overall decreasing tendency in occupancy rates. This decreasing trend was statistically significant only for Umurbey Reservoir ($Z = -2.14$, $p = 0.033$; Sen's slope = −2.45), while Tayfur Reservoir showed a near-significant trend ($Z = -1.79$, $p = 0.074$) (Table 3). Comparable findings were reported by Zulkiflie et al. (2024), who similarly concluded that precipitation alone is insufficient to explain reservoir storage variability. In a study conducted by Serifoglu Yilmaz (2025) in Artvin Province, Türkiye, reported non-significant trends ($p > 0.05$) using Mann–Kendall analysis, supporting the interpretation that observed variations may not represent statistically robust trends within comparable study periods.

In addition to hydroclimatic factors, reservoir occupancy is influenced by site-specific characteristics such as inflow variability, storage capacity, and water use patterns. Therefore, reservoirs should be evaluated individually. In this study, Bray–Curtis similarity and cluster analyses revealed clear differences among reservoirs based on their occupancy dynamics (Table 6, Figure 4). According to the similarity matrix, a main group consisting of Gökçeada, Atikhisar, Ayvacık, Umurbey, and Tayfur reservoirs exhibited relatively high similarity values (generally above 0.80), indicating comparable temporal occupancy patterns (Table 6). In contrast, Bakacak reservoir showed consistently lower similarity values with other reservoirs (approximately 0.49–0.56), clearly distinguishing it as the more divergent system in terms of occupancy behavior. Taşoluk reservoir exhibited intermediate similarity levels (approximately 0.59–0.67), suggesting partial separation from the main group but not as distinct as Bakacak (Table 6). These patterns should be interpreted as descriptive groupings derived from occupancy based similarity rather than direct evidence of causal relationships. However, the clustering structure is directly supported by both the similarity matrix (Table 6) and dendrogram (Figure 4), indicating that reservoirs may exhibit different temporal responses despite being subjected to similar hydroclimatic conditions. This variability likely reflects reservoir specific characteristics such as inflow variability, storage capacity, water demand, and operational practices.

A similar pattern was observed in the interannual similarity analysis (Table 5, Figure 3), where certain years (e.g., 2013–2014: 93.95% and 2015–2019: 91.84%) exhibited high similarity, whereas others (e.g., 2021–2023: 63.80%) showed markedly lower similarity. This indicates that both temporal variability and reservoir-specific dynamics contribute to the observed clustering patterns.

Overall, these findings indicate that reservoir occupancy rates are characterized by weak and mostly statistically insignificant decreasing tendencies. This suggests that reservoir occupancy is influenced not only by hydroclimatic variability within the study period but also by interannual hydrological variability, water demand, and reservoir operation practices. In addition, drought conditions, defined as deviations from average precipitation levels, may significantly affect agriculture, ecosystems, and water resources (Li et al., 2021). Increasing variability between certain years may indicate growing sensitivity of reservoir systems to climatic fluctuations and water use pressures.

Therefore, reservoir dynamics in the region are likely governed by multiple interacting factors, including hydroclimatic variability, evaporation losses, water abstraction, and reservoir-specific operational conditions. However, these factors were not directly quantified in this study, which represents an important limitation. Nevertheless, the findings provide a useful basis for future studies, which may benefit from incorporating additional variables and alternative analytical approaches to achieve more comprehensive and robust results.

CONCLUSION

This study examined temporal variability in reservoir occupancy rates across seven reservoirs in Çanakkale Province between 2013 and 2023**.** The main contribution of this study is the integrated application of trend analysis (Mann–Kendall and Sen's slope), correlation analysis (Spearman's rank), and similarity-based clustering (Bray–Curtis) to evaluate reservoir dynamics at a regional scale.

The results showed that reservoir occupancy rates varied between 2.7% and 79.2% during the study period. Mann–Kendall analysis indicated that no statistically significant trends were observed for most reservoirs ($p > 0.05$), whereas a statistically significant decreasing trend was detected only for Umurbey Reservoir ($Z = -2.14$, $p = 0.033$; Sen's slope = -2.45). In addition, Spearman's correlation analysis revealed no statistically significant relationships between annual precipitation and reservoir occupancy rates ($p > 0.05$), despite moderate correlation coefficients in some reservoirs. Overall, the results indicate that reservoir dynamics in the region are shaped by multiple interacting influences rather than a single controlling factor, such as annual precipitation alone.

In this context, it is recommended that sustainable water resource management strategies be strengthened at both regional and local levels. The findings demonstrate that management strategies should not rely solely on annual precipitation but should also consider temporal variability, reservoir specific dynamics, and water demand patterns. Integrated planning that considers climatic variability, reservoir operation policies, and demand management is crucial for ensuring sustainable water security over time. Stable reservoir levels are important not only for water supply but also for aquatic ecosystems. Variations in occupancy may affect habitat conditions and water availability. Therefore, sustainable reservoir management and consideration of climatic variability are important for maintaining ecological balance.

Increasing public awareness of water conservation and encouraging responsible water use may help reduce pressure on reservoir systems. In the context of projected changes in precipitation and drought frequency in Türkiye, adaptive management approaches may become increasingly important for regional sustainability.

This study has some limitations. The analysis was based on annual reservoir occupancy and precipitation data. Operational variables specific to each reservoir (e.g., water withdrawals, release regimes, and evaporation) were not explicitly considered. In addition, the study period represents a limited temporal scale for detecting long term hydroclimatic trends. Future studies should incorporate higher temporal resolution data and additional hydrological and management-related variables to provide a more comprehensive assessment of reservoir dynamics.

ACKNOWLEDGEMENTS

The author would like to thank the General Directorate of State Hydraulic Works in Türkiye for their support in sharing the water occupancy rates data used within the scope of the study. Furthermore, the author thanks the Provincial Directorate of Agriculture and Forestry for providing access to the provincial level information reports containing precipitation data compiled by the General Directorate of Meteorology of Türkiye.

Compliance with Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

The author confirms that the data supporting the findings of this study are available within the article.

AI Disclosure

AI-assisted technology was not used in the preparation of this work, except for grammar and spelling checks.

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Acta Nat. Sci. (2026) 7(1): 65-73
DOI: 10.61326/actanatsci.v7i1.463
e-ISSN: 2718-0638

Original Research Article
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ACTA NATURA ET SCIENTIA
PRENSIP

Body Performance Indices of the Invasive Red Cornetfish (*Fistularia petimba* Lacepède, 1803) in Taşucu Bay, Türkiye

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Please cite this paper as follows:
Bozkaya, S., Taşkavak, E., & Gürkan, Ş. (2026). Body Performance Indices of the Invasive Red Cornetfish (*Fistularia petimba* Lacepède, 1803) in Taşucu Bay, Türkiye. *Acta Natura et Scientia*, *7*(1), 65-73. https://doi.org/10.61326/actanatsci.v7i1.463

ARTICLE INFO

Article History
Received: 04.02.2026
Revised: 15.10.2026
Accepted: 16.10.2026
Available online: 12.06.2026

Keywords:
Red cornetfish
Length-weight relationship
Condition factor
Hepatosomatic index
Eastern Mediterranean

ABSTRACT

The red cornetfish (*Fistularia petimba* Lacepède, 1803) is an invasive fish species commonly found along the eastern Mediterranean coast of Türkiye. This study examined the length-weight relationship (LWR), condition factor (Fulton-K), and hepatosomatic index (HSI) of *F. petimba* specimens by sex. Between 2021 and 2024, 306 individuals (♀: 224, ♂: 56, juvenile: 26) were collected as bycatch from commercial fishing vessels in Taşucu Bay, Mersin Province. The sex ratio was 1:0.3 (♀:♂). The length-weight relationship was $W = 0.0002 \times L^{3.289}$ ($r^2 = 0.952$) for all individuals, $W = 0.0002 \times L^{3.222}$ ($r^2 = 0.949$) for females, and $W = 7E\text{-}05 \times L^{3.514}$ ($r^2 = 0.951$) for males. Positive allometric growth was detected. Fulton-K condition factor values were 0.49±0.03 for all individuals, 0.50±0.03 for females, and 0.47±0.06 for males. Hepatosomatic index (HSI) values were 0.85±0.05 for all individuals, 0.94±0.06 for females, and 0.57±0.08 for males. The data from this study on the red cornetfish population in Taşucu Bay, Mersin Province, may contribute significantly to understanding the body dynamics of the species.

INTRODUCTION

The eastern Mediterranean coasts of Türkiye have long served as critical habitats for numerous Lessepsian vertebrate and invertebrate species, providing essential areas for feeding and reproduction. Invasive fish species exert significant impacts on ecosystems by altering biodiversity through competition, predation, hybridization, and displacement of native species (Bariche et al., 2009). This process places considerable pressure on the ecological balance of the Mediterranean and directly affects the population dynamics of native species (Galil et al., 2019).

The red cornetfish, *Fistularia petimba* Lacepède, 1803, is an invasive species in the family Fistulariidae found in coastal and benthic regions. This benthopelagic carnivore feeds primarily on small fishes and invertebrates, playing a notable role in trophic networks (Bozkaya et al., 2023). *F. petimba* ranges from the Atlantic coasts (eastern and western) to the Indo-Pacific region, inhabiting sandy bottoms, coral reefs, and seagrass beds (Bray, 2023). The species was first recorded in the Mediterranean in the early 2000s and has since been increasingly reported along Turkish coasts (Ünlüoğlu et al., 2018; Çiftçi et al., 2019; Cerim et al., 2021; Tsaousi & Kalogirou, 2023). Additional eastern Mediterranean records include the Syrian coast (Hussein et al., 2019) and Oman Sea (Yasemi, 2012), with sightings also documented in Atlantic waters (Bañón & Sande, 2008). Recent biodiversity compilations confirm its continued expansion in the Mediterranean basin (Dragičević et al., 2019; Kovačić et al., 2021).

Although the red cornetfish has no commercial value, it is frequently caught as bycatch in fishing nets. It is currently listed as "Least Concern (LC)" on the IUCN Red List (Carpenter et al., 2015). Its expanding distribution is documented in various regional checklists and biodiversity reports (Bañón & Sande, 2008; Dragičević et al., 2019; Kovačić et al., 2021). Research on the species has focused on length–weight relationships, condition factors, hepatosomatic indices, and morphometric and meristic characteristics, aiming to better understand its population dynamics (Azevedo et al., 2004; Dias et al., 2014; Carassou et al., 2017; Ergüden et al., 2023). These studies highlight the species' adaptation to the Mediterranean and its potential ecological impacts.

Regarding *F. petimba*, most studies conducted in the Mediterranean and elsewhere have focused on occurrence records, while relatively few have examined growth values (b). Existing studies suggest that *F. petimba* may exhibit positive allometric growth (b>3) across a wide range of marine environments, from the Brazilian coast to the Pacific Ocean and the south-eastern Mediterranean coasts (Letourneur et al., 1998; Dias et al., 2014; Bozkaya et al., 2023; Ergüden et al., 2023; Papageorgiou et al., 2023). Recent studies show that the rapid spread of *F. petimba* in the Mediterranean may increase competition with native species and alter ecosystem services (Bozkaya et al., 2023; Papageorgiou et al., 2023). Studies along the eastern Mediterranean coasts of Türkiye offer valuable insights into the condition and energy reserves of local populations (Çiftçi et al., 2019; Ragheb, 2022). This study examines the length–weight relationship (LWR), condition factor (Fulton's K), and hepatosomatic index (HSI) of *F. petimba* individuals in Taşucu Bay by sex. The findings may help clarify the species' body dynamics and its role in the Mediterranean ecosystem.

MATERIAL AND METHODS

In this study, 306 *F. petimba* specimens were collected as bycatch from commercial trawl vessels operating along Türkiye's eastern Mediterranean coast (Taşucu Bay) across four seasons between 2021 and 2024.The sample consisted of 224 females, 56 males, and 26 juveniles (Figure 1). Specimens were transported to the laboratory under cold chain conditions to ensure tissue integrity and subsequently dissected. Prior to dissection, total lengths (TL, cm, excluding filament) were measured using a standard fish measuring ruler, while total body weights (W, g) and internal organ weights (g) were recorded with a precision balance accurate to 0.01 g. Sex determination was performed macroscopically during dissection, following established ichthyological protocols (Lagler et al., 1977).

The length–weight relationship (LWR) was calculated using the following equation (Eq. 1) (Pauly, 1983):

$$W = aL^b \quad (1)$$

where W represents body weight (g), L is total length (cm), a is the intercept, and b is the slope. Parameters a and b were estimated after log10 transformation of the data, as recommended for linearization of exponential growth models (Froese, 2006). A slope value (b) equal to 3 indicates isometric growth, values below 3 indicate negative allometry, and values above 3 indicate positive allometry (Ricker, 1975; Froese, 2006). The length-weight (L-W) relationship of the population was estimated separately for females, males, and all individuals combined. Additionally, the standard error and confidence intervals for the parameter b, derived from the length and weight measurements, were calculated using the Student's t-test. Analysis of covariance

Figure 1. *Fistularia petimba* Lacepède, 1803 (Photo: Simge Bozkaya)

Figure 2. Photographic documentation of liver tissue structure in red cornet fish specimens

(ANCOVA) was employed to test for statistically significant differences in the length-weight relationships between groups. The hepatosomatic index (HSI) was calculated using the following formula (Eq. 2) (Sulistyo et al., 2000):

$$HSI = 100 \times \frac{liver\ weight\ (g)}{body\ weight\ (g)} \quad (2)$$

HSI is widely used as an indicator of energy reserves and metabolic activity in fish, reflecting both nutritional status and environmental stress (Lambert & Dutil, 1997; Lloret et al., 2014).

The condition factor (Fulton's K) was calculated using the following equation (Eq. 3) (Ricker, 1979):

$$Fulton's\ K = \frac{100 \times W(g)}{L^3} \quad (3)$$

Fulton's K is a traditional measure of fish well-being and robustness, often applied to assess population health and habitat suitability (Froese, 2006).

All statistical analyses were performed using Statistica 12.5, SPSS 26 and Microsoft Excel. Differences between sexes in HSI and Fulton-K were tested using the Student's t-test, with statistical significance set at $p < 0.05$. The use of parametric tests for biometric indices is consistent with previous studies on fish population dynamics (Sokal & Rohlf, 2012).

RESULTS

The length–weight (LW) relationship was calculated for 306 specimens examined in this study (female: 224, male: 56, immature: 26). The LW relationship was computed separately for females, males, and all individuals combined. For the entire sample, the relationship was $W=0.0002\times L^{3.289}$ ($r^2=0.952$; 95% CI: 3.289–0.117). For females, the equation was $W=0.0002\times L^{3.222}$ ($r^2=0.949$; 95% CI: 3.221–0.146), while for males it was $W=7\times10{-}5\times L^{3.514}$ ($r^2=0.951$; 95% CI: 3.514–0.212). Data analysis revealed positive allometric growth across all categories examined (Figure 3).

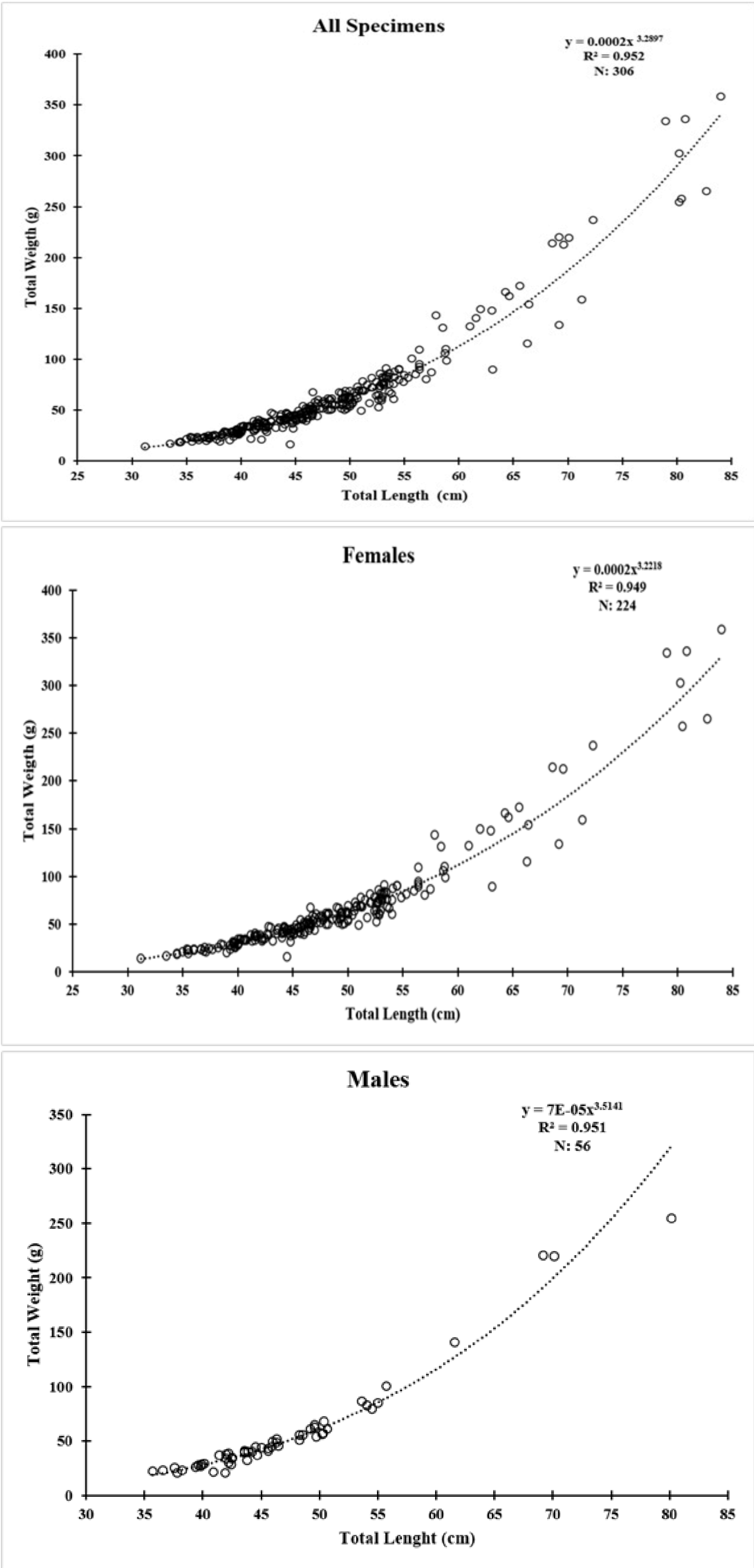

Figure 3. Graphical representations illustrating the length-weight relationship (LWR) among individual specimens

Table 1. Calculated biological index data presented as mean values with standard error (Mean±SE) and corresponding statistical difference ($p < 0.05$)

Biological Index	Female	Male	Combined Sex	p
Condition Factor (Fulton-K)	0.50±0.03	0.47±0.06	0.49±0.03	p <0.05*
Hepatosomatic Index (HSI)	0.94±0.06	0.57±0.08	0.85±0.05	p <0.05*

Note: *: *statically differences*

The t-test indicated that both sexes exhibited positive allometric growth, with no statistically significant differences in their LW relationships. In this study, we examined the effect of sex on body weight (W, g) using analysis of covariance (ANCOVA), controlling for total length (TL, cm). Of the 280 individuals analyzed, 56 were male and 224 were female. Descriptive statistics showed that mean female weight (65.95 ± 53.79 g) was higher than mean male weight (56.52 ± 47.59 g). ANCOVA results indicated that total length had an extremely strong, statistically significant effect on weight ($F_{1,277} = 1842.11$; $p < 0.001$). In contrast, after controlling for total length, sex did not have a significant effect on weight ($F_{1,277} = 0.022$; $p = 0.882$). Parameter estimates showed that a 1 cm increase in total length corresponded to an average 5.41 g increase in weight (B = 5.411; 95% CI: 5.162–5.659). However, the weight difference for males compared with females of the same length was not statistically significant (B = 0.424; $p = 0.882$). The model showed high explanatory power: 87% of the variance in weight was explained by total length and sex ($R^2 = 0.870$; adjusted $R^2 = 0.869$). These findings indicate that the raw weight differences between sexes are driven not by sex itself, but by differences in length among individuals. In conclusion, total length is the primary determinant of weight in the studied population, and there is no significant difference in weight between females and males of the same length.

Fulton's K condition values were examined for individuals distributed throughout the region. Immature individuals were not included in the Fulton's K calculation. The analysis revealed that female individuals exhibited a higher mean condition value when compared to their male counterparts (♀: 0.50 ± 0.03; ♂: 0.47 ± 0.06). Statistical analysis demonstrated that this observed difference between the sexes was statistically significant (*t*-test = 2.36, $p = 0.018$, $p < 0.05$).

In determining hepatosomatic index values, only sexes were considered; index values for immature individuals were not evaluated. Hepatosomatic index (HSI) values were calculated and analyzed, and the results demonstrated that female individuals had a considerably higher mean HSI value when compared to male individuals (♀: 0.94±0.06; ♂: 0.57±0.08). Statistical testing confirmed that this observed difference between the sexes was statistically significant (*t*-test = 3.57, $p = 0.0004$, $p < 0.05$).

Table 1 comprehensively presents and summarizes the Fulton-K condition factor values and HSI hepatosomatic index values that were obtained from this study, along with the statistical differences that were observed between the male and female sexes.

DISCUSSION

This study determined length–weight relationship (LWR), condition factor (Fulton-K), and hepatosomatic index (HSI) values for *F. petimba*, an invasive species along Türkiye's Eastern Mediterranean coast. We calculated values by sex and for all individuals combined, providing insights into growth dynamics, energy allocation, and reproductive physiology. The length–weight relationship is a key indicator for assessing fish growth patterns, biological status, and energy allocation. It is widely used to understand population dynamics in teleost fish (Tesh, 1971; Ricker, 1975; Wootton, 1998; Froese, 2006). Our LWR values showed positive allometric growth for all individuals, females, and males. This means body weight increases faster than body length, suggesting sex-specific differences in growth characteristics. In fish, the length–weight relationship is influenced by factors such as season, habitat, gonad maturity, sex, nutrition and stomach fullness, condition, the number of specimens collected, and sampling frequency (Tesh, 1971; Wootton, 1998). Therefore, differences in length–weight relationships among studies are thought to result from one or more of these factors acting together.

Positive allometry has been reported in other invasive species in the Mediterranean, such as *Fistularia commersonii* and *Siganus rivulatus*, reflecting adaptive strategies in novel ecosystems (Harmelin-Vivien et al., 2005; Bariche et al., 2009; Kalogirou, 2010). The findings align with values reported for *F. petimba* along the coasts of Cyprus and the Eastern Mediterranean (Bozkaya et al., 2023; Ergüden et al., 2023; Papageorgiou et al., 2023). Similar growth patterns have also been observed in populations from the Atlantic and Indo-Pacific regions (Azevedo et al., 2004; Dias et al., 2014), suggesting that environmental conditions, prey availability, and reproductive cycles strongly influence growth trajectories.

Body condition levels in the study area differed significantly between sexes. Females exhibited higher condition values than males, likely explained by reproductive metabolism and energy storage strategies, as females allocate more energy to gonadal development (Lambert & Dutil, 1997; Lloret et al., 2014). The study's mean condition factor (0.49±0.03) indicates moderate energy status and overall health for the species. These results are consistent with values reported by Bozkaya et al. (2023) (0.47±0.06) and Ergüden et al. (2023) (0.52±0.05). However, a population in India showed higher values (0.99±0.04) (Suyani et al., 2024). Such differences may result from geographic location, environmental conditions, prey availability, and sampling period (Adams et al., 1993). Seasonal variation in condition factor has been widely documented in teleosts, with higher values often associated with pre-spawning periods when energy reserves are maximized (Wootton, 1998).

HSI serves as an important biological indicator reflecting the liver's energy storage capacity and overall metabolic status. It varies depending on glycogen and lipid accumulation in liver tissue (Sulistyo et al., 2000). Some pelagic species store glycogen primarily in muscle tissues due to continuous swimming activity (Timur, 2006), but benthopelagic species such as *F. petimba* rely heavily on liver reserves. In this study, HSI values showed significant sex-based differences. Female individuals exhibited higher HSI values, primarily due to reproductive cycles and vitellogenin synthesis during spawning (Blazer, 2002; Wootton, 1998). During vitellogenesis, females store lipids and glycogen in the liver to support egg development, while males lack this process. Elevated HSI values in females are also influenced by sex hormones such as estrogen, which regulate liver metabolism (Love, 1980; Mommsen & Walsh, 1988).

Feeding strategies, seasonal variation, and environmental conditions further amplify these sex-based differences (Adams et al., 1993; Lloret et al., 2014). When comparing our results with those of Bozkaya et al. (2023), differences were observed despite both studies being conducted in the same region. These discrepancies may be attributed to sampling season, reproductive stage, and nutritional status of individuals at the time of collection.

CONCLUSION

As a conclusion, the comprehensive data obtained from this study on *F. petimba* populations inhabiting the Eastern Mediterranean coastal waters provide valuable insights into the species' body dynamics and physiological characteristics. By integrating LWR, Fulton-K, and HSI analyses, this research contributes to understanding the adaptive strategies of invasive fishes in the Mediterranean and highlights the importance of sex-specific physiological assessments in invasion ecology.

ACKNOWLEDGEMENTS

We thank Paşa Balıkçılık and the crew of the Paşa Fishing Boat for their assistance during fieldwork. This work is partly based on the first author's MSc thesis.

Compliance with Ethical Standards

Authors' Contributions

SB: Conceptualization, Methodology, Data curation, Writing – original draft

ET: Investigation, Resources, Writing – original draft, Writing – review & editing

ŞG: Conceptualization, Methodology, Data curation, Formal analysis

All authors critically reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. This work is partly based on the first author's MSc thesis.

AI Disclosure

The authors declare that no generative artificial intelligence was used in creating images, graphs, tables, corresponding titles, or in writing this article.

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Acta Nat. Sci. (2026) 7(1): 74-84
DOI: 10.61326/actanatsci.v7i1.491
e-ISSN: 2718-0638

Original Research Article
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www.actanatsci.com

ACTA NATURA ET SCIENTIA PRENSIP

LC-MS/MS-Based Phenolic Profiling of Flaxseed (*Linum usitatissimum* L.) and Safflower (*Carthamus tinctorius* L.) Seed Extracts and Their Antibacterial Activity Against Selected Fish Pathogenic Bacteria

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Please cite this paper as follows:
Saed, M. A. R., Karga, M., Kenanoğlu, O. N., & Bilen, S. (2026). LC-MS/MS-Based Phenolic Profiling of Flaxseed (*Linum usitatissimum* L.) and Safflower (*Carthamus tinctorius* L.) Seed Extracts and Their Antibacterial Activity Against Selected Fish Pathogenic Bacteria. *Acta Natura et Scientia*, *7*(1), 74-84. https://doi.org/10.61326/actanatsci.v7i1.491

ARTICLE INFO

Article History
Received: 18.05.2026
Revised: 12.06.2026
Accepted: 23.06.2026
Available online: 24.06.2026

Keywords:
Antibacterial activity
Aquaculture
Broth microdilution
Fish pathogens
LC-MS/MS
MIC

ABSTRACT

The growing demand for sustainable disease-control strategies in aquaculture has increased interest in plant-derived compounds with potential antibacterial activity against fish pathogens. This study investigated the LC-MS/MS-based phenolic profile and *in vitro* antibacterial activity of aqueous methanolic flaxseed (*Linum usitatissimum* L.) and safflower (*Carthamus tinctorius* L.) seed extracts against selected fish pathogenic bacteria. The extracts were obtained using 40% methanol, concentrated, and prepared as aqueous stock solutions. The stock concentrations were 0.148 g mL^{-1} for flaxseed and 0.101 g mL^{-1} for safflower. The phenolic profiles of the final aqueous stock solutions were then determined by LC-MS/MS and expressed as μg L^{-1} (ppb). LC-MS/MS analysis showed that the flaxseed extract was mainly characterized by a tannic acid-rich profile, together with trans-ferulic acid, caffeic acid, gallic acid, quercetin, rutin trihydrate, cinnamic acid, and 2,5-dihydroxybenzoic acid. In contrast, the safflower seed extract contained trans-ferulic acid, cinnamic acid, 2,5-dihydroxybenzoic acid, ellagic acid, caffeic acid, and quercetin as the detected phenolic constituents. Antibacterial activity was tested by the broth microdilution method against *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Pseudomonas putida*, *Yersinia ruckeri*, and *Vibrio anguillarum*. The strongest antibacterial activity was observed for flaxseed extract against *A. hydrophila* and *A. salmonicida*, with MIC values of 25 and 50 μg mL^{-1}, respectively. Safflower seed extract showed its highest inhibitory activity against *A. salmonicida*, with an MIC value of 50 μg mL^{-1}, while higher MIC values were recorded against *A. hydrophila*, *Y. ruckeri*, and *V. anguillarum*. No inhibitory activity was detected against *P. putida* for either extract within the tested concentration range. These results indicate that aqueous methanolic flaxseed and safflower seed extracts, particularly flaxseed, have species-dependent antibacterial activity against fish pathogens. Although these findings suggest that the tested extracts may be considered as preliminary natural antibacterial candidates for aquaculture-related research, further studies are needed to evaluate their safety, stability, and *in vivo* applicability.

INTRODUCTION

Bacterial infections remain one of the persistent constraints in intensive aquaculture, where both opportunistic and primary pathogens can cause mortality, reduced growth, treatment costs, and economic losses in farmed fish. Species belonging to *Aeromonas*, *Yersinia*, *Vibrio*, and *Pseudomonas* are frequently associated with bacterial diseases in freshwater, marine, and brackish-water fish culture (Tobback et al., 2007; Öztürk & Altınok, 2014; Terzi et al., 2023). Among these bacteria, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Yersinia ruckeri*, *Vibrio anguillarum*, and *Pseudomonas putida* are particularly relevant to fish health studies because of their association with disease outbreaks and opportunistic infections under culture conditions. Antibiotics are still used for the treatment and control of bacterial diseases in aquaculture. However, their repeated or inappropriate application may contribute to antimicrobial resistance, environmental contamination, and residue-related concerns (Ramesh & Souissi, 2018; Terzi & Isler, 2019). These limitations have encouraged the evaluation of non-antibiotic strategies, including vaccines, probiotics, bacteriophages, organic acids, and plant-derived bioactive products that may support disease control in aquaculture (Van Hai, 2015; Tadese et al., 2022; Li et al., 2022). Medicinal and aromatic plants are important sources of phenolic and other bioactive compounds, including phenolic acids, flavonoids, tannins, terpenoids, and alkaloids. These compounds have been associated with antibacterial, antioxidant, anti-inflammatory, and immunomodulatory activities, although their effects may vary according to compound type, concentration, extraction method, and target organism (Van Hai, 2015; Bouarab-Chibane et al., 2019; Tadese et al., 2022). In antibacterial screening studies, however, crude plant extracts need to be interpreted with caution, since their activity is rarely explained by a single constituent. Rather, the observed response may result from combined interactions, including additive, synergistic, or antagonistic effects among several compounds in the extract (Liu, 2003; Terzi et al., 2023). Despite the growing body of research on plant-derived antibacterials in aquaculture, the antibacterial potential of oilseed extracts, particularly flaxseed (*Linum usitatissimum* L.) and safflower (*Carthamus tinctorius* L.), against multiple fish pathogens remains insufficiently explored. Flaxseed is known for its nutritional value and phenolic constituents, including phenolic acids, lignans, flavonoids, and tannin-related compounds, whereas safflower is valued for its seed oil and phytochemical composition. Evaluating the phenolic composition and strain-specific antibacterial activity of these seed extracts may therefore contribute to the identification of candidate plant-derived products for aquaculture-related research.

Although a number of studies have evaluated plant extracts against fish pathogens, comparative investigations that combine targeted phenolic profiling with antibacterial screening remain relatively scarce and provide a stronger basis for selecting candidate extracts for aquaculture-related research (Bilen et al., 2016; Karga et al., 2020; Metin et al., 2021; Pires et al., 2021; Li et al., 2022). Chemical characterization is particularly important because it helps relate the observed antibacterial activity to the phytochemical composition of the extract, rather than interpreting inhibition data alone. The present study aimed to determine the phenolic composition of aqueous methanolic flaxseed and safflower seed extracts using LC-MS/MS and to evaluate their *in vitro* antibacterial activity against selected fish pathogenic bacteria using the broth microdilution method.

MATERIAL AND METHODS

Seed Materials and Extract Preparation

Seeds of safflower and flaxseed were purchased from a commercial herbal supplier in Kastamonu, Türkiye. The seed materials were transported to the laboratory under dry conditions and stored at room temperature until extraction. Before extraction, the seeds were cleaned manually to remove foreign materials and then ground into a fine powder using a laboratory grinder.

For each plant, 50 g of dried seed powder was extracted with 1 L of 40% methanol (v/v) for 72 h at room temperature. During extraction, the mixtures were inverted twice daily. After extraction, the mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated to dryness under reduced pressure using a rotary evaporator. The remaining dry extract residue was dissolved in 30 mL of distilled water. The final aqueous stock extract concentrations were determined gravimetrically as 0.101 g mL^{-1} for safflower seed and 0.148 g mL^{-1} for flaxseed. Based on these gravimetrically determined stock concentrations, the amount of dry extract obtained from 50 g of seed powder was calculated as 3.03 g for safflower seed and 4.44 g for flaxseed, corresponding to extraction yields of 6.06% and 8.88%, respectively.

LC-MS/MS Analysis of Phenolic Compounds

The phenolic composition of the extracts was determined at Kastamonu University Central Research Laboratory using a Shimadzu LCMS-8040 triple quadrupole mass spectrometer system. The injection volume was 10 µL. Chromatographic separation was performed on an Inertsil ODS-4 column (3 µm, 2.1 × 50 mm). Mobile phase A consisted of water containing 0.1% formic acid, and mobile phase B consisted of methanol containing 0.1% formic acid. The flow rate was 0.4 mL min^{-1}, and the column temperature was maintained at 40°C. The gradient program was applied as follows: 5% A and 95% B from 4.00 to 7.00 min, followed by a shift to 95% A and 5% B at 7.01 min, which was maintained until the end of the run at 12.00 min.

The monitored phenolic compounds included catechin, tannic acid, myricetin, naringenin, ellagic acid, quercetin, luteolin, chrysin, cinnamic acid, caffeic acid, trans-ferulic acid, 2,5-dihydroxybenzoic acid, rutin trihydrate, and gallic acid. The corresponding precursor/product ion transitions are presented in Table 1. Results were expressed as µg L^{-1} (ppb) for the final aqueous extract solutions. The LC-MS/MS analysis was performed as a targeted phenolic profiling analysis using the available laboratory method parameters, including chromatographic conditions, ionization modes, and precursor/product ion transitions. A full validation dataset including LOD, LOQ, recovery, and precision values was not available for all monitored phenolic compounds. Therefore, the LC-MS/MS results were interpreted as targeted chemical profiling data for the final aqueous stock extract solutions rather than as fully validated absolute quantification.

Table 1. LC-MS/MS ion transitions used for the determination of phenolic compounds in the extracts

Compound	Ionization mode	Precursor > product ions (m/z)
Catechin	Positive	291.1 > 138.90, 122.90
Tannic acid	Negative	182.90 > 123.60, 78.20
Myricetin	Negative	316.80 > 178.90, 151, 137
Naringenin	Negative	270.80 > 150.90, 118.90, 92.90
Ellagic acid	Negative	300.80 > 229.10, 257.10
Quercetin	Negative	300.80 > 150.80, 121.10, 106.90
Luteolin	Negative	284.80 > 217, 198.80, 174.90
Chrysin	Negative	252.80 > 142.90, 119, 209.10, 106.90
Cinnamic acid	Positive	149.10 > 130.90, 103.20
Caffeic acid	Negative	178.90 > 135, 134, 89
trans-Ferulic acid	Negative	192.80 > 132.90, 178.00
2,5-Dihydroxybenzoic acid	Negative	153.10 > 107.90, 109.00
Rutin trihydrate	Negative	609 > 300, 271, 301
Gallic acid	Negative	168.80 > 124.90, 78.90, 81.00

Bacterial Strains

The antibacterial activity of the extracts was tested against five fish pathogenic bacteria: *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Pseudomonas putida*, *Yersinia ruckeri*, and *Vibrio anguillarum*. The bacterial strains had been previously isolated from diseased rainbow trout (*Oncorhynchus mykiss* Walbaum) and identified using conventional morphological and biochemical analyses, together with 16S rRNA gene sequencing (Terzi et al., 2023). For the present antibacterial assays, the isolates were revived from stock cultures and used at the first passage after subculture on Mueller Hinton Agar. The plates were incubated at 25°C for 24–48 h until colonies developed. Bacterial suspensions were prepared in sterile Mueller Hinton Broth and first adjusted to the turbidity of a 0.5 McFarland standard. The suspensions were then diluted in sterile Mueller Hinton Broth to obtain a final inoculum concentration of approximately 10^6 CFU mL^{-1} for use in the microdilution assay.

Minimum Inhibitory Concentration Determination

The minimum inhibitory concentration (MIC) values of the extracts were determined using the broth microdilution method, in accordance with the general principles of antimicrobial susceptibility testing outlined by the Clinical and Laboratory Standards Institute (CLSI, 2018), with minor adaptations for plant extracts. Serial dilutions of safflower seed and flaxseed extracts were prepared in Mueller Hinton Broth in sterile 96-well microplates to obtain final extract concentrations ranging from 25 to 1600 μg mL^{-1}. Each well contained the appropriate extract dilution and the standardized bacterial suspension. Wells containing bacterial suspension without extract served as growth controls, whereas wells containing broth without bacterial inoculum served as sterility controls. The plates were incubated at 25°C for 48 h. The MIC was defined as the lowest extract concentration that visibly inhibited bacterial growth after incubation (CLSI, 2018; Hajji et al., 2010; Terzi et al., 2023). All assays were performed in triplicate, and MIC values were expressed as μg mL^{-1}.

Data Reporting

LC-MS/MS results were expressed as μg L^{-1} (ppb) for the final aqueous stock extract solutions. The LC-MS/MS analysis of each final aqueous stock extract solution was performed as a single analytical measurement; therefore, standard deviation values could not be calculated. The results were presented descriptively as targeted phenolic profiling data. Compounds not detected were reported as "nd" (not detected) (Table 2). MIC values were presented descriptively, as MIC represents an endpoint dilution value obtained from serial dilution assays and corresponds to discrete intervals; therefore, no inferential statistical analysis was applied to the MIC endpoints.

RESULTS

Phenolic Composition of the Extracts

LC-MS/MS analysis showed that the two extracts had distinct phenolic profiles (Table 2). In the safflower seed extract, trans-ferulic acid was detected at the highest concentration (590.54 ppb), followed by cinnamic acid (183.04 ppb), 2,5-dihydroxybenzoic acid (154.22 ppb), ellagic acid (63.45 ppb), caffeic acid (19.38 ppb), and quercetin (4.73 ppb). Rutin trihydrate, gallic acid, and tannic acid were not detected in this extract. The flaxseed extract showed a broader phenolic profile. Tannic acid was the dominant compound, with a concentration of 25596.31 ppb. Considering the gravimetrically determined flaxseed stock extract concentration of 0.148 g mL^{-1}, the detected tannic acid concentration corresponds to approximately 0.017% of the total dry extract in the stock solution. Other detected compounds included trans-ferulic acid (761.81 ppb), caffeic acid (183.60 ppb), gallic acid (142.52 ppb), quercetin (79.26 ppb), 2,5-dihydroxybenzoic acid (63.75 ppb), rutin trihydrate (33.79 ppb), and cinnamic acid (12.44 ppb). Ellagic acid was not detected in the flaxseed extract. Catechin, myricetin, naringenin, luteolin, and chrysin were not detected in either extract. Overall, the flaxseed extract contained a greater number of detected phenolic compounds, and tannic acid was the dominant compound within the monitored phenolic panel.

Table 2. Comparative phenolic composition µg L^{-1} (ppb) of aqueous methanolic seed extracts from flaxseed and safflower determined by LC-MS/MS.

Compound	Safflower	Flaxseed
Cinnamic acid	183.04	12.44
Caffeic acid	19.38	183.60
trans-Ferulic acid	590.54	761.81
Rutin trihydrate	nd	33.79
Quercetin	4.73	79.26
Ellagic acid	63.45	nd
Gallic acid	nd	142.52
Tannic acid	nd	25596.31
2,5-Dihydroxybenzoic acid	154.22	63.75

***Note:** Values are expressed as µg L^{-1} (ppb) and represent single LC-MS/MS measurements of the final aqueous stock extract solutions; therefore, standard deviation values are not presented. nd: not detected. Compounds monitored but not detected in either extract: catechin, myricetin, naringenin, luteolin, and chrysin.

Antibacterial Activity of the Extracts

The extracts showed species-dependent inhibitory activity against the tested fish pathogenic bacteria (Table 3). Flaxseed extract exhibited the lowest MIC values recorded in the study, with MICs of 25 µg mL^{-1} against *A. hydrophila*, 50 µg mL^{-1} against *A. salmonicida*, and 400 µg mL^{-1} against *Y. ruckeri*. No inhibitory activity was detected against *P. putida* or *V. anguillarum* within the tested concentration range. Safflower seed extract showed an MIC value of 50 µg mL^{-1} against *A. salmonicida*. Higher MIC values were recorded against *A. hydrophila* (400 µg mL^{-1}), *Y. ruckeri* (800 µg mL^{-1}), and *V. anguillarum* (1600 µg mL^{-1}), whereas no inhibition was observed against *P. putida*. Among the tested pathogens, *A. hydrophila* was the most susceptible bacterium to flaxseed extract, while *A. salmonicida* was inhibited by both extracts at 50 µg mL^{-1}. In contrast, *P. putida* showed no detectable susceptibility to either extract under the tested conditions.

Table 3. MIC values of aqueous methanolic safflower and flaxseed seed extracts against selected fish pathogenic bacteria

Bacteria	Safflower	Flaxseed
A. hydrophila	400	25
A. salmonicida	50	50
P. putida	NI	NI
Y. ruckeri	800	400
V. anguillarum	1600	NI

Note: Values are expressed as µg mL^{-1}. MIC: minimum inhibitory concentration; NI: no inhibition detected within the tested concentration range.

DISCUSSION

The present study demonstrated that aqueous methanolic flaxseed and safflower seed extracts have detectable *in vitro* antibacterial activity against selected fish pathogenic bacteria. The activity was not uniform across bacterial species, indicating that the antibacterial response was influenced by both the extract type and the target microorganism. This pattern is consistent with the behavior of crude plant extracts, whose effects may vary depending on their phenolic composition, extraction characteristics, and the susceptibility of the tested bacterium (Bouarab-Chibane et al., 2019; Li et al., 2022).

A key finding was the strong activity of flaxseed extract against *A. hydrophila* and *A. salmonicida*, with MIC values of 25 and 50 µg mL^{-1}, respectively. These bacteria are important in aquaculture because *Aeromonas* species are associated with economically relevant bacterial diseases in cultured fish. In particular, *A. hydrophila* has been linked to motile aeromonad septicemia, hemorrhagic septicemia, and ulcerative or red-sore disease, whereas *A. salmonicida* is recognized as the causative agent of furunculosis and septicemic infections, especially in salmonid fish (Öztürk & Altınok, 2014; Dallaire-Dufresne et al., 2014; Semwal et al., 2023). In addition, *A. hydrophila* has been widely used in experimental challenge models to evaluate the protective potential of plant-derived products in rainbow trout (Bilen et al., 2016).

Therefore, the low MIC values recorded for flaxseed extract are particularly relevant as preliminary *in vitro* antibacterial evidence.

The more pronounced antibacterial activity of flaxseed extract may be partly associated with its broader targeted phenolic profile. LC-MS/MS analysis showed that tannic acid was the dominant compound within the monitored phenolic panel of this extract, together with trans-ferulic acid, caffeic acid, gallic acid, quercetin, rutin trihydrate, cinnamic acid, and 2,5-dihydroxybenzoic acid. Phenolic compounds can interact with bacterial cell surfaces and may affect microbial growth through various mechanisms, including membrane disruption and enzyme inhibition (Bouarab-Chibane et al., 2019). Tannins, in particular, have been reported to inhibit bacterial growth through iron chelation, interference with cell wall synthesis, disruption of membrane integrity, and inhibition of certain metabolic pathways (Farha et al., 2020). The predominance of tannic acid among the monitored phenolic compounds may therefore be associated with the distinct chemical profile of flaxseed extract; however, this relationship should not be interpreted as evidence that tannic acid alone was responsible for the observed antibacterial activity. In addition, the apparently high tannic acid value should be interpreted in relation to the concentrated final stock extract solution and the total dry extract concentration, rather than as a direct concentration in the original seed material.

However, the antibacterial effect should not be attributed to tannic acid alone. Although the high tannic acid concentration in flaxseed extract may have contributed to the lower MIC values, crude plant extracts represent complex chemical mixtures rather than single-compound preparations. Moreover, the LC-MS/MS values reported in the present study represent the concentrations of selected phenolic compounds in the final aqueous stock solutions, whereas the MIC values are expressed as total dry extract concentrations. Therefore, the quantified phenolics should be interpreted primarily as chemical markers of the extract profile, rather than as direct evidence that the measured individual compounds reached antibacterial concentrations in the MIC wells. The observed inhibition may also involve unmonitored constituents of the crude extract and additive or synergistic interactions among multiple compounds. Liu (2003) emphasized that the biological effects of plant-derived products are often associated with combined phytochemical interactions rather than isolated compounds alone. Similarly, Bouarab-Chibane et al. (2019) reported that the antibacterial activity of polyphenols depends on multiple physicochemical properties and interactions with bacterial cell surfaces. In the present study, flaxseed extract contained not only tannic acid but also trans-ferulic acid, caffeic acid, gallic acid, quercetin, rutin trihydrate, cinnamic acid, and 2,5-dihydroxybenzoic acid. These phenolic acids, flavonoids, and tannin-related compounds may have contributed to the observed antibacterial effect through complementary mechanisms, including membrane disturbance, enzyme inhibition, metal chelation, and interference with bacterial metabolic processes (Cushnie & Lamb, 2005; Bouarab-Chibane et al., 2019; Farha et al., 2020). Flavonoids such as quercetin and rutin have also been associated with antimicrobial activity in previous reviews (Cushnie & Lamb, 2005). Therefore, the stronger activity of flaxseed extract against *A. hydrophila* and *A. salmonicida* should be interpreted as the possible result of its overall phenolic profile, rather than as a direct effect of tannic acid alone.

The phenolic composition of the extracts was also compared with previous reports on flaxseed and safflower. Flaxseed has been reported to contain several phenolic acids and flavonoids, including ferulic acid, cinnamic acid derivatives, rutin, quercetin, and related phenolic constituents (Oomah et al., 1995; Koçak, 2024). In agreement with these reports, the present flaxseed extract contained trans-ferulic acid, caffeic acid, gallic acid, quercetin, rutin trihydrate, cinnamic acid, and 2,5-dihydroxybenzoic acid. Notably, tannic acid was detected as the dominant compound within the monitored phenolic panel, distinguishing flaxseed extract from safflower seed extract. For safflower seed, previous studies have reported phenolic acids and flavonoids such as trans-ferulic acid, quercetin derivatives, rutin, luteolin, naringin, and catechin-type compounds (Yu et al., 2013). In the present study, safflower seed extract was mainly characterized by trans-ferulic acid, cinnamic acid, 2,5-dihydroxybenzoic acid, ellagic acid, caffeic acid, and quercetin. The detection of trans-ferulic acid as the main compound in the present safflower seed extract is in line with previous reports showing that safflower seeds contain ferulic acid and related hydroxycinnamic acid-derived phenolic constituents, including N-feruloylserotonin and N-(p-coumaroyl)serotonin (Kim et al., 2007; Katsuda et al., 2009; Yu et al., 2013). Nevertheless, direct numerical comparison with the literature should be made cautiously because the present LC-MS/MS results were expressed as μg L^{-1} (ppb) in the final aqueous stock solutions, whereas many previous studies report total phenolics as mg GAE g^{-1} or individual compounds as mg g^{-1} dry material or extract. Therefore, differences among studies may reflect plant material and variety, extraction solvent, sample preparation, target analyte panel, analytical method, and unit expression. For this reason, the present results are more appropriately compared with previous studies in terms of detected compound groups and relative compositional trends rather than direct absolute concentrations. Within this context, the tannic acid-rich and broader targeted phenolic profile of flaxseed extract may be associated with its lower MIC values compared with safflower seed extract; however, this association should not be interpreted as direct evidence of a single-compound effect.

The safflower seed extract showed a different inhibitory pattern. Its lowest MIC value was observed against *A. salmonicida* (50 μg mL^{-1}), which was comparable to the value recorded for flaxseed extract against the same pathogen. Higher concentrations were required to inhibit *A. hydrophila*, *Y. ruckeri*, and *V. anguillarum*. The absence of tannic acid and the generally lower detected levels of several phenolic compounds compared with flaxseed extract may partly account for the higher MIC values observed for safflower seed extract. However, this interpretation should also be considered associative, since purified compounds and compound combinations were not tested in the present study.

Both extracts inhibited *Y. ruckeri*, although the MIC values were higher than those recorded for the more susceptible *Aeromonas* species. *Y. ruckeri*, the causative agent of enteric redmouth disease, remains an important pathogen in salmonid aquaculture (Tobback et al., 2007). The MIC values of 400 μg mL^{-1} for flaxseed and 800 μg mL^{-1} for safflower seed indicate modest but detectable inhibitory activity against this pathogen. These results justify further screening against *Y. ruckeri*, but they also suggest that extract concentration, formulation, and exposure conditions would need to be optimized before any aquaculture-related application is considered.

No inhibitory activity was detected against *P. putida* within the tested concentration range. Members of the genus *Pseudomonas* are known for variable antimicrobial susceptibility, and resistance-related traits in this group may involve mechanisms such as reduced membrane permeability and efflux-mediated tolerance (Pang et al., 2019). Therefore, the lack of inhibition against *P. putida* should be interpreted as a species- or isolate-dependent response. Together with the MIC differences observed among the other bacteria, this finding supports the view that the antibacterial activity of the extracts was selective rather than uniformly broad-spectrum.

The activity against *V. anguillarum* was weak compared with the activity observed against the *Aeromonas* species. Only the safflower seed extract inhibited this pathogen, and this inhibition was recorded at a relatively high MIC value of 1600 μg mL^{-1}. *V. anguillarum* is an important fish pathogen and the causative agent of vibriosis, particularly in marine and brackish-water aquaculture (Frans et al., 2011). The lower susceptibility of *V. anguillarum* in the present study may reflect a species-dependent response to the tested extracts. Safflower seed extract should not be considered a strong candidate against *V. anguillarum* at this stage, although further work on extract concentration, formulation, and exposure conditions may clarify its potential.

When compared with previous plant-derived antibacterial studies against fish pathogens, the MIC values obtained in the present study fall within a broad activity range, but clear extract- and pathogen-dependent differences are evident. Karga et al. (2020) reported a lower MIC value for *Laurus nobilis* aqueous methanolic extract against *A. hydrophila* (3.125 μg mL^{-1}) than those observed in the present study for flaxseed and safflower extracts against the same pathogen (25 and 400 μg mL^{-1}, respectively). In the same study, *Brassica nigra* extract inhibited *V. anguillarum* at 100 μg mL^{-1}, which was lower than the MIC value recorded for safflower seed extract against *V. anguillarum* in the present study (1600 μg mL^{-1}). In contrast, Terzi et al. (2023) reported considerably higher MIC values for *Vaccinium arctostaphylos* aqueous methanolic extract against *A. hydrophila, A. salmonicida, Y. ruckeri*, and *P. putida* (8.75 mg mL^{-1}; equivalent to 8750 μg mL^{-1}), indicating that the present flaxseed extract showed stronger inhibitory activity against *A. hydrophila, A. salmonicida,* and *Y. ruckeri.* Similarly, Pires et al. (2021) reported MIC values ranging from 400 to 1600 μg mL^{-1} for Maclura tinctoria heartwood extract against Aeromonas strains, with an MIC of 400 μg mL^{-1} against one *A. hydrophila* strain. Therefore, the flaxseed extract in the present study, with an MIC of 25 μg mL^{-1} against *A. hydrophila*, appeared more active than several previously reported plant extracts, whereas safflower seed extract showed moderate or weaker activity depending on the target bacterium. Overall, these comparisons indicate that flaxseed extract showed relatively strong activity against *Aeromonas* species within the context of plant-derived antibacterial studies, whereas safflower seed extract showed moderate to weak activity, particularly against *Y. ruckeri* and *V. anguillarum*. However, direct comparison among studies should be made cautiously because extraction solvent, extract type, bacterial strain, assay conditions, and reporting units may differ.

A methodological point worth noting is that the extract composition was evaluated using LC-MS/MS-based targeted phenolic analysis, which provided ppb values for selected phenolic compounds in the final aqueous extract solutions. Unlike relative peak-area percentages commonly reported in GC-MS based plant extract studies, these quantitative data directly represent compound concentrations rather than chromatographic abundance. This distinction is important because the results reflect the targeted phenolic profile within the analyzed compound panel, rather than relative abundance based solely on detector response. Studies combining quantitative chemical profiling with antimicrobial screening have used comparable data to support the biological interpretation of plant extracts (Hajji et al., 2010; Terzi et al., 2023).

Since the present study was limited to *in vitro* antibacterial screening, the findings should be regarded as preliminary evidence. Another limitation of the present study is that full LC-MS/MS validation parameters, including LOD, LOQ, recovery, and precision values, were not available for all monitored phenolic compounds. Therefore, the LC-MS/MS results should be considered as targeted phenolic profiling data supporting comparison between the extracts, rather than as fully validated absolute quantification. Further *in vivo* studies are required to evaluate the safe concentration range, formulation suitability, and biological effects of the extracts in fish. Additional bactericidal and compound-level assays would also help clarify the mechanisms underlying the observed inhibitory activity. Despite these limitations, the findings provide relevant preliminary evidence that aqueous methanolic seed extracts of flaxseed and safflower seed have antibacterial potential against selected fish pathogens. The notable inhibitory activity of flaxseed extract against *A. hydrophila* and *A. salmonicida*, together with its broader targeted phenolic profile, supports its further evaluation as a natural antibacterial candidate for aquaculture-related research.

CONCLUSION

Aqueous methanolic seed extracts of flaxseed and safflower showed species-dependent antibacterial activity against selected fish pathogenic bacteria. Flaxseed extract exhibited the strongest inhibitory activity, particularly against *Aeromonas hydrophila* and *Aeromonas salmonicida,* with MIC values of 25 and 50 μg mL^{-1}, respectively. Safflower seed extract also inhibited *A. salmonicida* at 50 μg mL^{-1}, although higher MIC values were required against the other susceptible pathogens. LC-MS/MS analysis showed that flaxseed extract had a broader detected phenolic profile and was distinguished by the presence of tannic acid as the dominant monitored phenolic compound, whereas safflower seed extract was mainly characterized by trans-ferulic acid, cinnamic acid, 2,5-dihydroxybenzoic acid, and ellagic acid. The broader targeted phenolic profile of flaxseed extract was associated with lower MIC values against some pathogens, particularly *A. hydrophila* and *Y. ruckeri*. However, the observed antibacterial activity should be interpreted as the response of the whole crude extract rather than as the direct effect of a single quantified phenolic compound. Further *in vivo* studies are needed to evaluate the safety, effective concentration range, formulation suitability, and practical potential of these extracts in fish health.

ACKNOWLEDGEMENTS

The authors would like to thank Assoc. Prof. Dr. Ertuğrul TERZİ for kindly providing the fish pathogenic bacterial strains used in this study.

Compliance with Ethical Standards

Authors' Contributions

MARS: Conceptualization, Investigation

MK: Methodology, Writing original draft

ONK: Writing original draft, Formal analysis

SB: Supervision, Writing – review & editing

All authors critically reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

Ethical approval was not required for this study because no live animals were used. The study was conducted using bacterial isolates and plant extracts under in vitro laboratory conditions.

Funding

Not applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

ChatGPT (GPT-5.5 Thinking, OpenAI) was used for language editing and structural improvement of the manuscript. The authors reviewed and validated all outputs and take full responsibility for the final content.

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Acta Nat. Sci. (2026) 7(1): 85-95
DOI: 10.61326/actanatsci.v7i1.479
e-ISSN: 2718-0638

Original Research Article
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ACTA NATURA ET SCIENTIA PRENSIP

Growth and Chlorophyll Responses of Wheat Seedlings to Putrescine Under PEG-Induced Drought Stress

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Please cite this paper as follows:
Şengül Toraman, P., Ergün, N., & Çalıcı, B. (2026). Growth and Chlorophyll Responses of Wheat Seedlings to Putrescine Under PEG-Induced Drought Stress. *Acta Natura et Scientia*, *7*(1), 85-95. https://doi.org/10.61326/actanatsci.v7i1.479

ARTICLE INFO

Article History
Received: 06.04.2026
Revised: 19.06.2026
Accepted: 24.06.2026
Available online: 27.06.2026

Keywords:

Gacer
Chlorophyll
PEG-6000
Putrescine
Triticum aestivum

ABSTRACT

Wheat is one of the most important cereal crops worldwide due to its broad cultivation area, diverse uses, and economic importance. This study aimed to determine the shoot length, shoot fresh and dry weight, root length, root fresh and dry weight, chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl a+b) amounts of wheat seedlings. The interactions of 5% polyethylene glycol (PEG-6000) and different putrescine (PUT) doses (0.5 mM and 1 mM) were examined in wheat (*Triticum turgidum* L. var. *dicoccum*, Gacer, *Triticum aestivum* L., Konya-2002) seedlings. Under the drought stress effect caused by PEG, two wheat varieties were negatively affected and a decrease in shoot length, fresh weight, dry weight and chlorophyll amounts was observed. In Gacer wheat, an increase in fresh root weight was observed under drought stress treated with PEG, while a decrease was observed in Konya-2002. Compared to the control, it was determined that the root length of Gacer wheat was less affected by the presence of PEG than that of Konya-2002. Compared to PEG, under the effect of PUT, increases were observed in shoot length, fresh weight, root length and fresh weight, chlorophyll a and total chlorophyll content in both wheat varieties. The applied 5% PEG-6000 created a drought stress effect in wheat and it was observed that PUT reduced this stress effect. When the parameters we used in our study are examined together, these findings suggest that Gacer wheat is more resilience to drought than Konya-2002.

INTRODUCTION

Cereals provide a significant portion of the protein and carbohydrate needs of humans. Therefore, wheat ranks first among the cultivated cereals all over the world (Doğru & Ergün, 2021). Türkiye, particularly Central Anatolia, has suitable agroecological conditions for wheat cultivation due to its geographical location, soil structure, and water resources, making the region an important center for crop production. Wheat is a vital part of rural life and remains the main cereal crop in the region. While

Türkiye ranks among the top wheat-producing countries globally, durum wheat is especially important as it is widely used for both human consumption and livestock feed, making its agricultural and industrial production highly significant (Pandey et al., 2015).

The conservation of local wheat genetic resources is important for agricultural sustainability, biodiversity, and food security (Özberk et al., 2016). Bread wheat varieties may be sensitive or resistant to various stresses with their differences (Atak, 2017). As a result of genetic changes of *Triticum turgidum* L., the ancestor of Emmer wheat, which was cultivated in the past, and tetraploid wheat used today, was formed. Today, it is cultivated as bulgur wheat in Germik, Çatalca, Develi, and given names belonging to the region where it is found. Gacer, known as emmer wheat (*Triticum turgidum* L. var. *dicoccum*) (2n=28), which is a wheat that is on the verge of being forgotten has a high nutritional value, especially with its very low gluten content, protein content varying between 17-20%, and high carotenoid content, is a wheat species that grows in Anatolia and whose population is decreasing (Bulut, 2016). Gacer wheat has recently started to be intensively cultivated again in Develi district of Kayseri province by our farmers and scientists who know its value. The Konya-2002 variety of *Triticum aestivum* (2n=42), another wheat species we used in our study, is known to be drought-sensitive, lodging-resistant, and resistant to winter and cold (Balkan & Gençtan, 2023). Drought stress is observed at the highest level in the world and will negatively affect plant production in the future. In addition, there will be a decrease of up to 80% in cereal production (Kadıoğlu, 2006; İlyas et al., 2021). The most important issue in breeding studies aimed at drought tolerance in plants is knowing the morphological and physiological response mechanisms that plants possess and use to overcome water deficiency and drought (Hussain et al., 2011).

Polyamines are widely found in living organisms (Kusano et al., 2008). It was initially thought that the main role of polyamines was that of direct protective molecules under stress conditions, and it was stated that they are compounds that are involved in a complex signaling system and have an important role in the regulation of stress tolerance. Some of these studies aim to prevent or eliminate the harmful effects of stresses that negatively affect plant development and yield in agricultural production through external applications (Hussain et al., 2011; Shu et al., 2012; Shi et al., 2013). However, it is stated that the physiological significance of this increase and the role of polyamines are still unclear, and that studies in this direction are necessary to understand the functions of polyamines in stress tolerance (Alcázar et al., 2006). Stress and its responses in plant adaptation are still a very important research topic. The study aimed to determine the effects of drought stress induced by 5% PEG-6000 on Gacer (*Triticum dicoccum* L.) and Konya-2002 (*Triticum aestivum* L.) wheat seedlings, to evaluate the role of different putrescine (PUT) doses on physiological parameters in mitigating stress conditions, and to reveal the responses of the Gacer variety, which has been researched to a limited extent in the literature, to drought stress.

MATERIAL AND METHODS

Two wheat genotypes were used in the experiment: Gacer (*Triticum turgidum* L. var. *dicoccum*) and Konya-2002 (*Triticum aestivum* L.). Gacer seeds were obtained after harvest from KAÇEM (Women Farmers Ecological Education and Production Center) in Kayseri, Türkiye, whereas Konya-2002 seeds were supplied by the Bahri Dağdaş International Agricultural Research Institute, Türkiye.

Before germination, seeds were surface-sterilized in 2% sodium hypochlorite for 20 min, rinsed thoroughly with distilled water, and soaked in distilled water for approximately 1 h. Seedlings were grown in 2-L plastic containers containing full-strength Hoagland nutrient solution (Arnon & Hoagland, 1940) adjusted to pH 5.7. The nutrient solution was renewed on the fifth day of growth.

When seedlings reached 10 days of age, polyethylene glycol and putrescine treatments were applied through the nutrient solution (Hellal et al., 2017). The experiment consisted of six treatment groups for each genotype: control, 5% PEG-6000, 0.5 mM putrescine, 1 mM putrescine, 5% PEG-6000 + 0.5 mM putrescine, and 5% PEG-6000 + 1 mM putrescine

(Money, 1989; Alobaidy, 2013; Çömlekçioğlu & Arıkan, 2017). At the end of the treatment period, the pots were divided into twelve different groups and shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight were measured. Dry weights were determined after oven-drying the plant material at 80°C for 48 h.

Chlorophyll a, chlorophyll b, and total chlorophyll contents were determined spectrophotometrically according to Porra et al. (1989).

Data Evaluation

Data were analyzed using three-way factorial analysis of variance (ANOVA), with genotype, PEG-6000 treatment, and putrescine concentration as fixed factors. When significant differences were detected, means were compared using Tukey post hoc test. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

When plant growth parameters were examined, a decrease in shoot length was observed in both PEG-treated wheat varieties compared to the control (Figure 1). While both wheat varieties showed a decrease in stem length under PEG-induced drought stress, the Gacer wheat variety was more affected in terms of stem elongation compared to the Konya-2002 variety. Putrescine doses increased stem lengths in both PEG-treated wheat varieties.

When plant growth parameters were examined, a decrease in root length was observed in both PEG-treated wheat varieties compared to the control (Figure 2). When comparing root length to the control with PEG application, it was observed that Gacer wheat was less affected than Konya-2002.

The decrease in the presence of PEG in Konya-2002 wheat compared to the control showed that it was more affected than Gacer. The pairwise comparison results of PUT doses were found to be significant ($p<0.001$). The significant difference between the doses was that the greatest increase in fresh weights in both wheats was observed in Konya-2002 with the 0.5 mM PUT application (Figure 3).

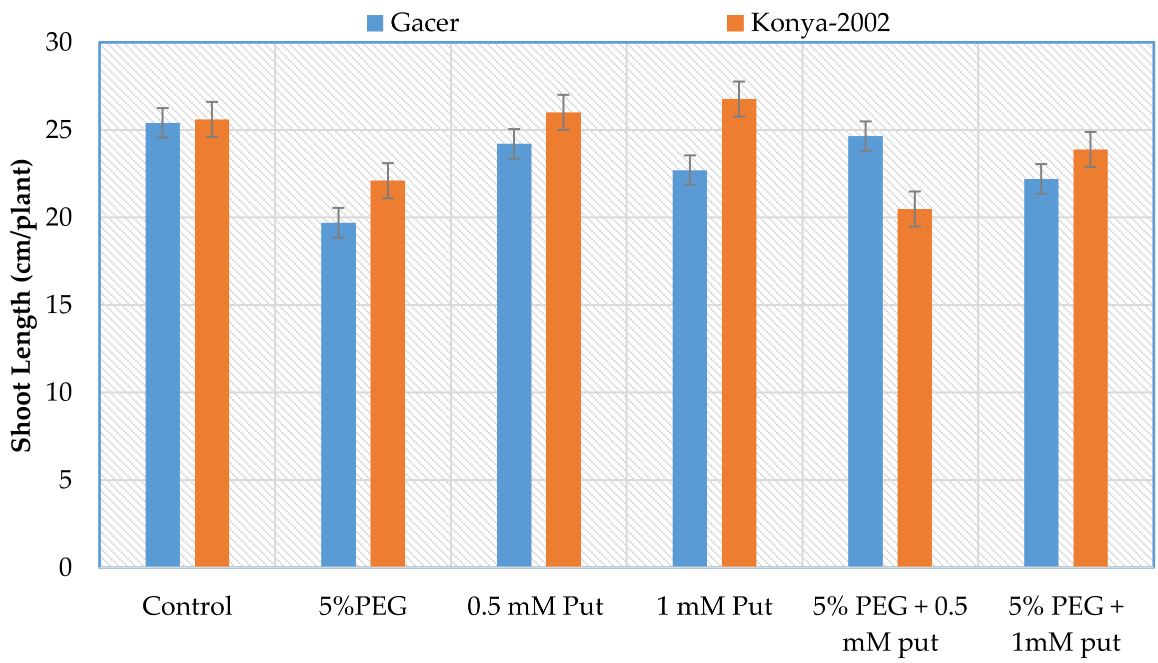

Figure 1. Changes in average shoot length of *Triticum dicoccum* 2n=28(Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions (Genotype = *p = 0.042), PEG (***p < 0.001), Putrescine (* p = 0.024), Genotype × PEG (**p < 0.001), Genotype × Putrescine (** p < 0.001), PEG × Putrescine (p = 0.063), Genotype × PEG × Putrescine (*p = 0.007).

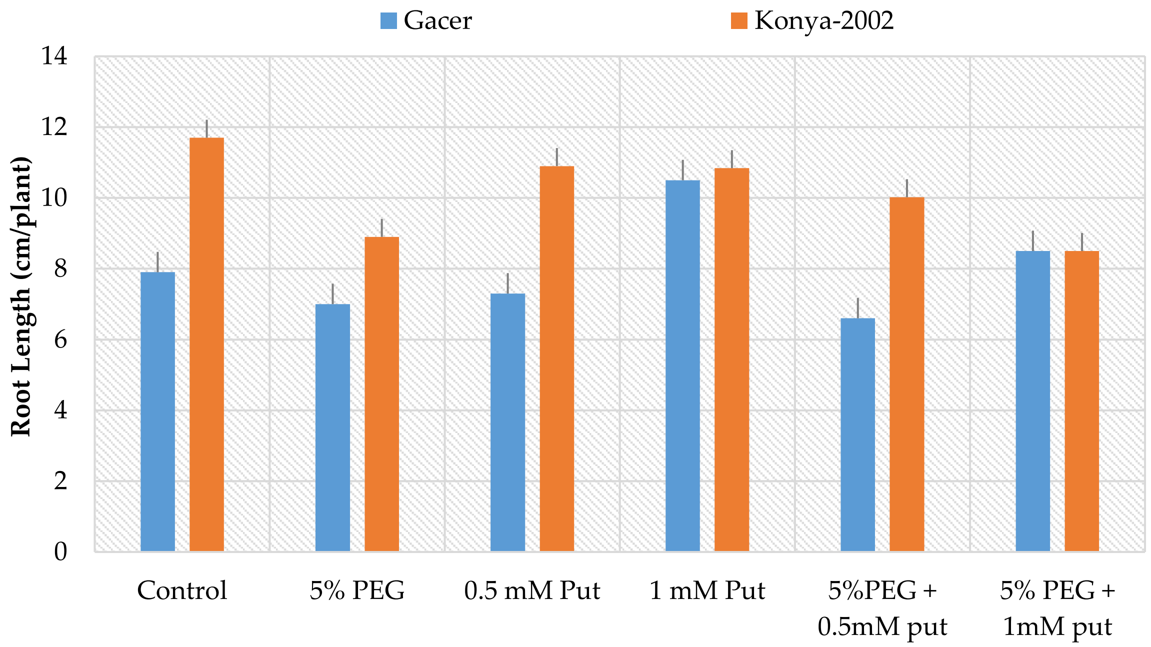

Figure 2. Changes in average root length of *Triticum dicoccum* 2n=28(Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions. Genotype (**p < 0.001), PEG (p** < 0.001), Putrescine (**p = 0.001), Genotype × PEG (* p = 0.020), Genotype × Putrescine (*p = 0.003), PEG × Putrescine (p = 0.490), Genotype × PEG × Putrescine (p = 0.795).

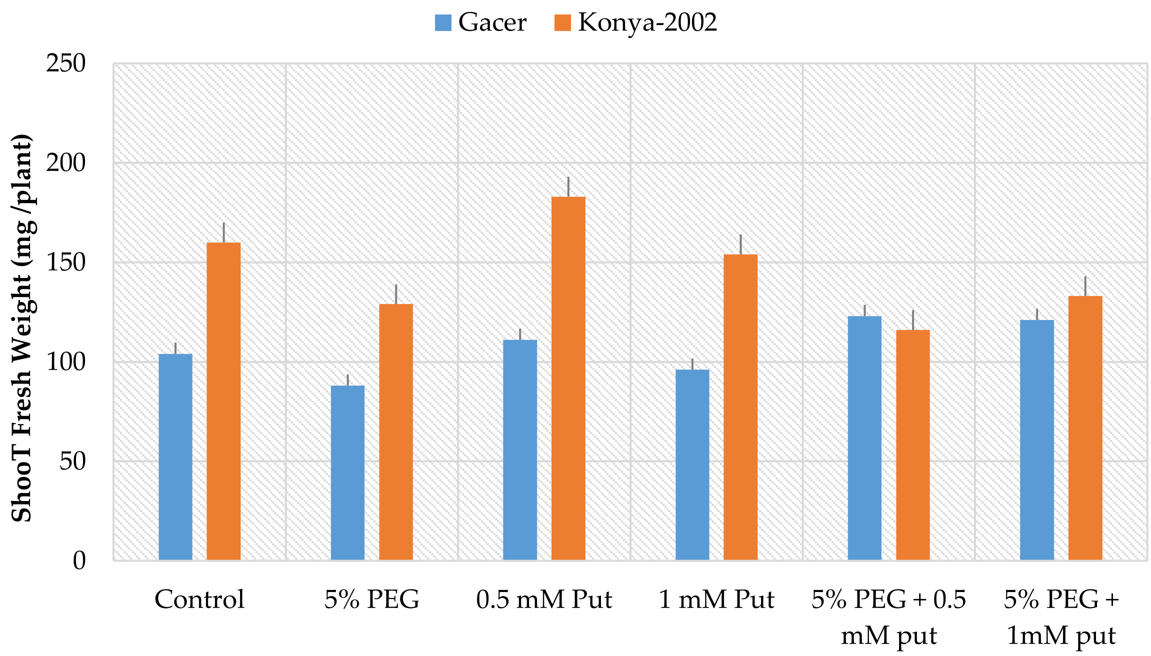

Figure 3. Changes in average shoot fresh weight of *Triticum dicoccum* 2n=28(Gacer) and *Triticum aestivum* 2n=42(Konya-2002) seedlings grown under PEG and PUT conditions. Genotype (p < 0.001), *PEG* (**p < 0.001), Putrescine (**p < 0.001), Genotype × PEG (*p = 0.022), Genotype × Putrescine (p = 0.491), PEG × Putrescine (*p = 0.03), Genotype × PEG × Putrescine (*p = 0.003).

PEG application resulted in a decrease in shoot dry weight in Gacer and Konya-2002 seedlings. PUT applications increased shoot dry weight in both wheat varieties. Increased PUT doses led to further increases in shoot dry weight. In shoot dry weight data, a decrease was observed in drought-affected Gacer and Konya-2002 wheat varieties with PEG application, while the combined application of PEG and Putrescine resulted in an increase in wheat ($p < 0.05$) (Figure 4).

Comparing the presence of PEG in the wheat to the control, an increase in root fresh weight was observed in Gacer, while a decrease was observed in Konya-2002. Root fresh weight increased proportionally with increasing PUT doses applied to Konya-2002. The greatest increase in root fresh weight in Gacer was observed with the 0.5 mM PUT application (Figure 5). The lowest average weight was observed in both wheat varieties with the PEG + 0.5 mM PUT application.

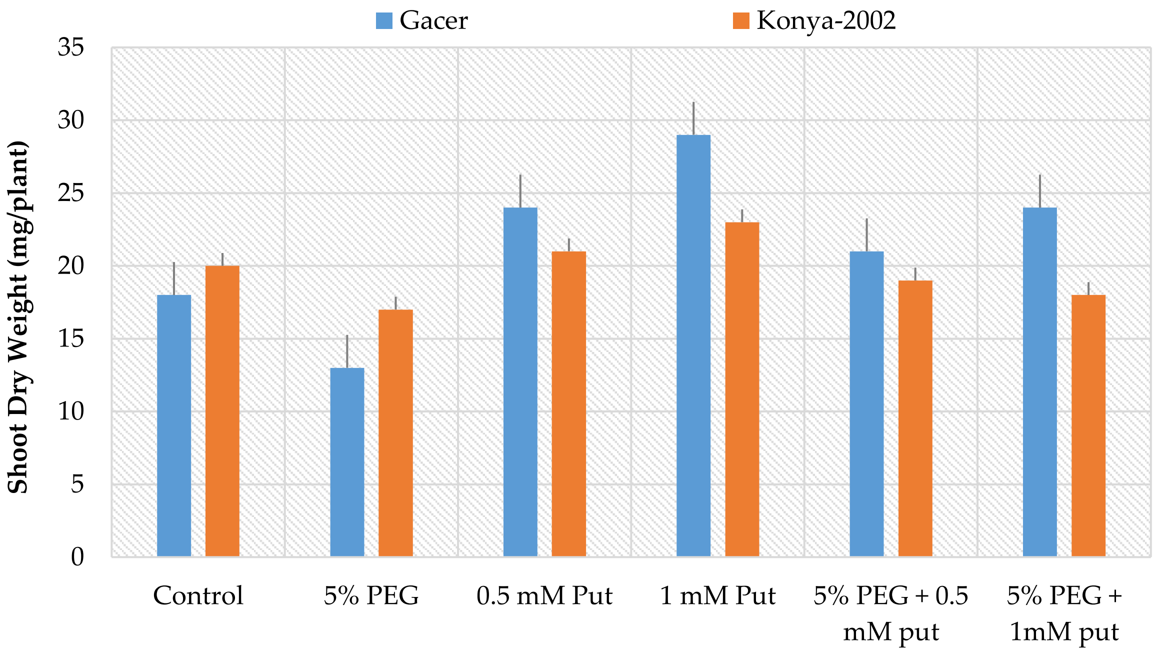

Figure 4. Changes in average shoot dry weight of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions. Genotype ($p = 0.513$), PEG (*$p = 0.009$), Putrescine (**$p < 0.001$), Genotype × PEG (*$p = 0.041$), Genotype × Putrescine ($p = 0.053$), PEG × PUT ($p = 0.542$), Genotype × PEG × Putrescine (**$p < 0.001$)).

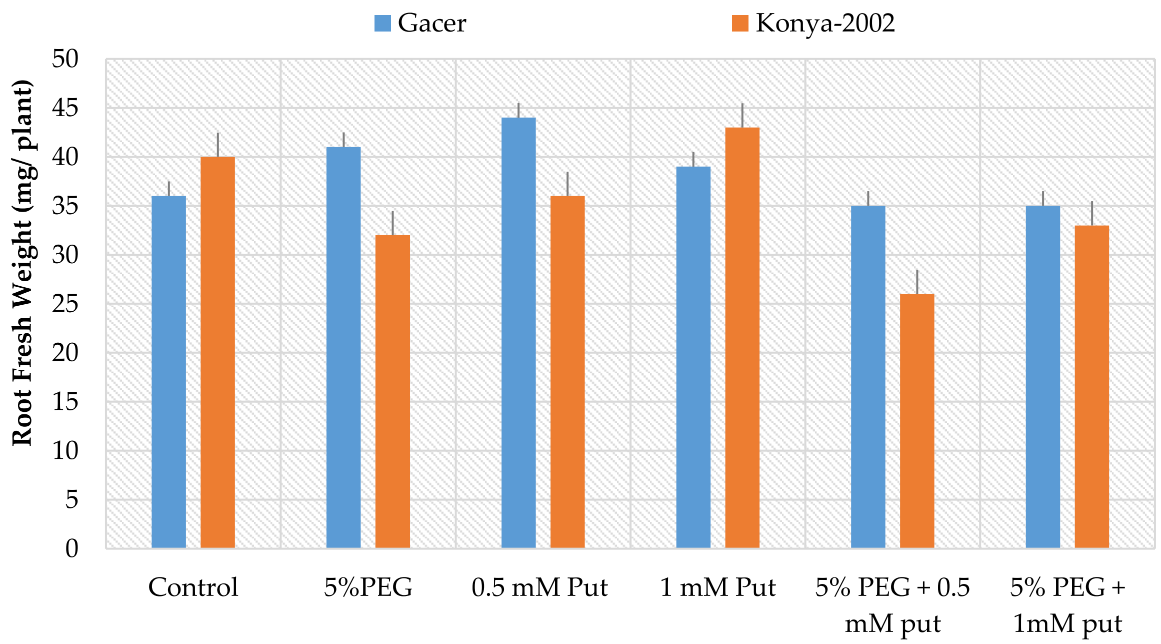

Figure 5. Changes in average root fresh weight of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions. Genotype (*$p = 0.034$), PEG ($p= 0.087$), PUT ($p < 0.001$), Genotype × PEG ($p = 0.298$), Genotype × PUT (**$p = 0.001$), PEG × PUT (*$p = 0.037$), Genotype × PEG × Putrescine ($p = 0.054$).

It was observed that the presence of PEG in wheat increased the dry root weight in Gacer and Konya-2002 compared to the control. The greatest increase was observed in Gacer. The least dry root weight was observed in Konya-2002 with the 0.5 mM PUT application. In Gacer, the greatest increase in root weight was observed with the 0.5 mM PUT application (Figure 6). In both wheats, the least average weight was observed with the control application.

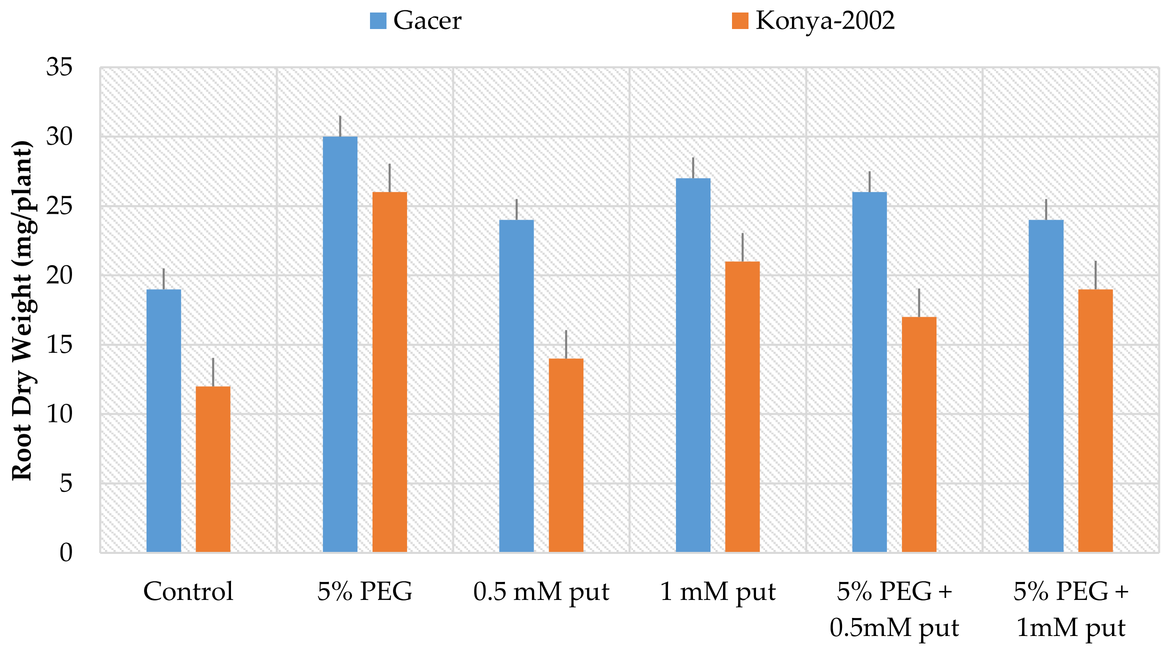

Figure 6. Changes in average root dry weight of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42(Konya-2002) seedlings grown under PEG and PUT conditions. (Genotype ($p < 0.001$), PEG ($p = 0.154$), PUT (**$p < 0.001$), Genotype × PEG ($p = 0.154$), Genotype × PUT ($p = 0.313$), PEG × PUT(**$p < 0.001$), Genotype × PEG × PUT (**$p < 0.001$).

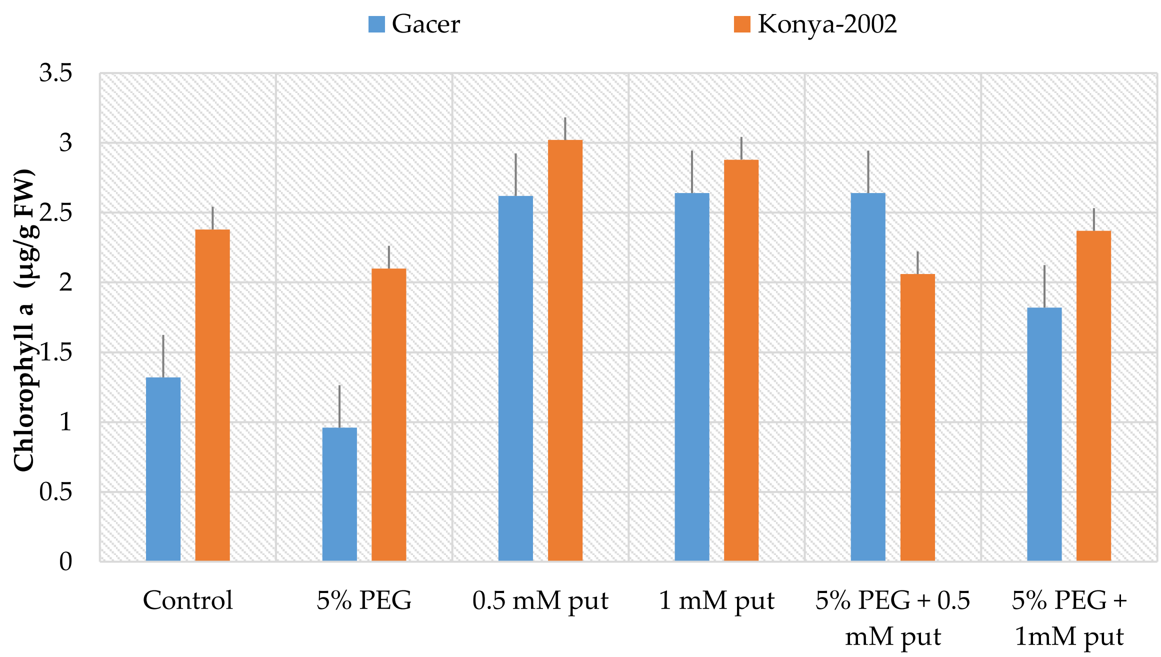

Figure 7. Changes in the average chlorophyll a content of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) grown under PEG and PUT conditions. **(**PEG (**, $p = 0.010$), Genotype ($p = 0.149$), PUT (**, $p = 0.009$).

Significant differences were found between the groups in terms of mean chlorophyll a (chl a) of Gacer and Konya-2002 wheat (p≤0.05). Compared to the control, PEG application resulted in a decrease in chlorophyll a and this decrease was most pronounced in Gacer (p≤0.05). The PUT application was found to significantly increase the amount of chlorophyll a in wheat. In the pairwise comparison, it was statistically significant that the greatest increase in chlorophyll a level (150%) was observed with the applied 0.5 mM PUT. (p≤0.05) (Figure 7).

Compared to the control, PEG application caused a decrease in chl b content in Gacer and Konya-2002 wheat varieties, and this decrease was statistically significant (p<0.001). The greatest decrease was observed in Konya-2002, at 37. 5%. In Gacer, PUT applications resulted in an increase in chl b content compared to the control, while in Konya-2002, a decrease was observed (Figure 8).

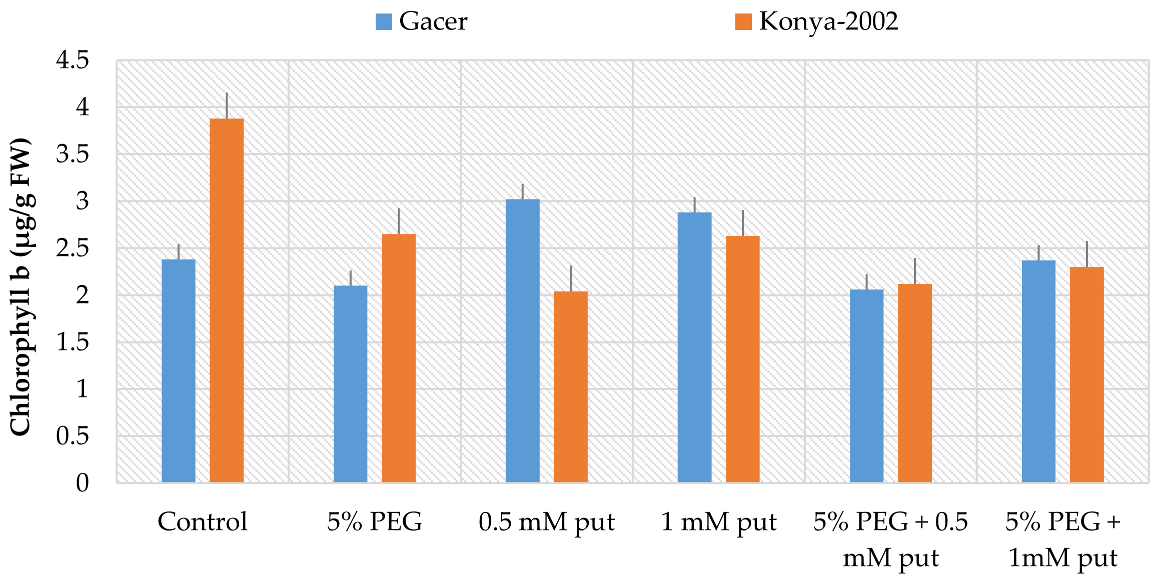

Figure 8. Changes in the average chlorophyll b content of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) grown under PEG and PUT conditions (PEG **, p < 0.001), Genotype (p = 0.298), Putrescine (p = 0.068).

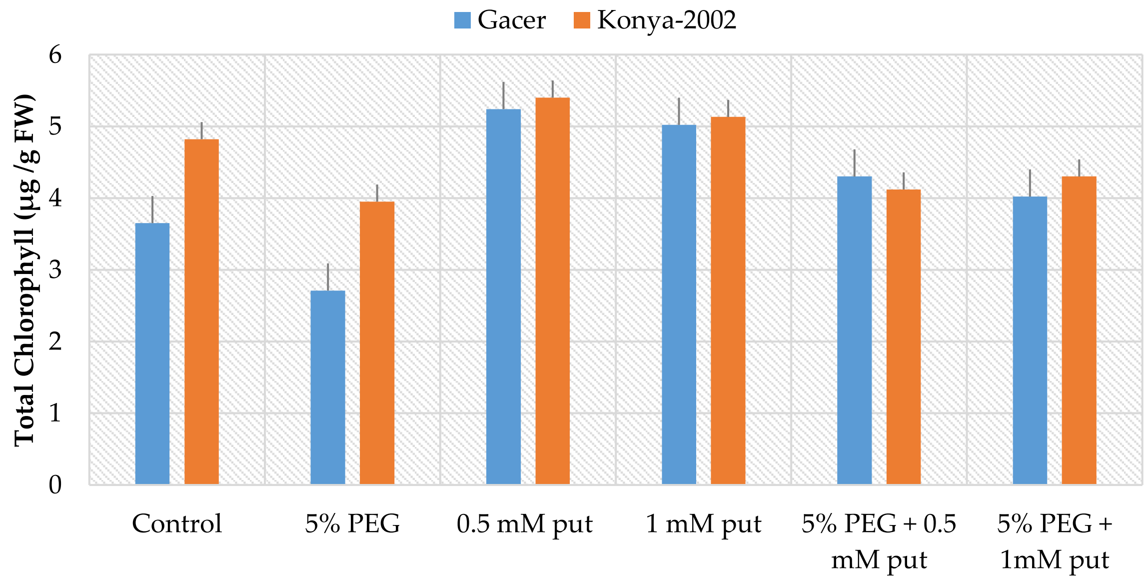

Figure 9. Changes in average total chlorophyll content of *Triticum dicoccum* 2n=28 (Gacer) and *Triticum aestivum* 2n=42 (Konya-2002) seedlings grown under PEG and PUT conditions. (**p < 0.001), Genotype (p = 0.098), PUT (*p = 0.048), Genotype × PUT (p = 0.065).

In Gacer wheat, the total chlorophyll content increased with the applied PUT doses, and no difference was observed between the doses. In Konya-2002 wheat, the greatest increase in total chlorophyll content was observed with 0.5 mM PUT and was found to be statistically significant ($p \leq 0.05$) (Figure 9).

DISCUSSION

The present study demonstrated that PEG-induced drought stress significantly affected growth and physiological traits in wheat, although the magnitude and direction of these effects varied depending on genotype and putrescine application. PEG treatment consistently reduced shoot growth, as well as chlorophyll content, confirming its effectiveness in simulating drought stress conditions. Similarly, İlhan et al. (2026) in their study determined that drought stress was induced in *Triticum dicoccum* Schrank and *Triticum aestivum* L. populations using different doses of PEG-6000, and that the measured values of shoot length, root length, shoot dry weight, root dry weight, shoot fresh weight, root fresh weight, and relative water content decreased as the PEG intensity increased. Zhong et al. (2025) supported our study that putrescine, used for therapeutic purposes and applied exogenously to the plant, reduced the stress effect in wheat. The decreased chlorophyll values of our drought-stressed wheat increased to high levels with putrescine. Bukhari et al. (2021) evaluated the effects of polyethylene glycol (PEG-6000) at osmotic potentials of −0.17, −0.32, −0.47, and −0.62 MPa on ten wheat genotypes. Their findings indicated that, compared with the control treatment, germination stress tolerance and root length tolerance were highest under the −0.62 MPa PEG treatment. Pekol et al. (2016) investigated the effects of drought and salinity stress on root and shoot growth in wheat species, including *Triticum turgidum* subsp., *Triticum aestivum*, and *Triticum monococcum* (einkorn wheat). According to the data obtained, they stated that *Triticum turgidum subsp. durum* wheat was more tolerant to salt and drought stresses than *T. aestivum* and *T. monococcum*.

With PEG application, a decrease in total chlorophyll content was observed in both wheat varieties, and Gacer wheat was the most affected with 64% ($p<0.001$). According to the pairwise comparison results, significant differences were found between control-PEG, control-PUT, PEG-PEG+PUT in Gacer and Konya-2002 wheat varieties ($p<0.001$). Rahman et al. (2024) reported that the application of 0.3 mM putrescine to wheat, rice and maize plants increased chlorophyll content and actual quantum yield in wheat, and this is consistent with our results. Balkan & Gençtan (2023) reported that chlorophyll content in the leaves of bread wheat varieties decreased with increasing levels of drought stress. Hebat-Allah et al. (2023) evaluated the interaction of different drought levels on wheat grains and the physiological characteristics of wheat treated with 3 doses (0.25, 0.5, and 1 mM) of putrescine. The study found that drought conditions reduced plant height, fresh and dry weights, while 1 mM putrescine application improved wheat growth performance under both control and drought conditions, which is consistent with our findings.

Alharby et al. (2021) reported that drought stress adversely affects plant growth, chlorophyll content, photosynthetic efficiency, and plant water status. They further demonstrated that seed pretreatment with polyamines and corn extract–enriched polyamines can enhance growth performance and yield-related parameters in wheat. Toraman et al. (2020) determined a decrease in chl a, chl b, chl a+b content, shoot length and dry weight in *Triticum aestivum* genotypes as a result of drought, flooding and salt stress applications. Abdelmoghny et al. (2020) found statistically significant differences in the morpho-physiological characteristics of *Gossypium hirsutum* under drought conditions. They stated that root length is very important in terms of cut leaf water loss and cell membrane permeability.

Importantly, the interaction effects revealed that the response to putrescine was not uniform across genotypes or stress conditions. Significant Genotype × PEG and Genotype × PEG × Putrescine interactions for several traits (shoot length, shoot dry weight, and root fresh weight) indicate that the effectiveness of putrescine is strongly genotype-dependent and modulated by stress intensity. This highlights that polyamine-mediated stress tolerance is a complex, multi-level physiological process rather than a simple additive effect.

The results indicate that Gacer wheat may be more drought-resistant than Konya-2002, as its root growth length is less affected by drought stress. The findings also confirm that putrescine plays a significant role in enhancing drought tolerance, although its effectiveness depends strongly on genotype and environmental stress context. These results have important implications for crop improvement strategies. The strong genotype-dependent response suggests that selection of drought-tolerant varieties such as Gacer, combined with exogenous application of polyamines, could be a promising strategy to improve wheat performance under water-limited conditions.

CONCLUSION

PEG-induced drought stress negatively affected growth and photosynthetic traits in both wheat genotypes; however, Gacer variety showed higher resilience to drought stress compared to the Konya-2002 variety. The fact that root growth of Gacer wheat was not significantly affected under PEG-induced drought stress conditions indicates that Gacer possesses more effective adaptation mechanisms to water deficiency. Putrescine application, particularly at 0.5 mM, effectively alleviated the detrimental effects of drought stress by improving growth parameters and chlorophyll content. These findings indicate that putrescine plays an important role in enhancing drought tolerance in wheat seedlings. In conclusion, the responses observed in this study demonstrate the superior drought resilience of Gacer and highlight the potential of putrescine as a stress-mitigating agent. Furthermore, the results support the conservation and wider utilization of Gacer, a nutritionally valuable local wheat genotype, in future wheat breeding and cultivation programs. Further molecular studies are required to elucidate the mechanisms underlying polyamine-mediated drought tolerance.

ACKNOWLEDGEMENTS

This article was derived from PhD thesis of the first author and funded by Scientific Research Projects Coordinator (BAP) of Hatay Mustafa Kemal University (21.D.028).

Compliance with Ethical Standards

Authors' Contributions

PŞT: Investigation, Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing

NE: Conceptualization, Project administration, Writing – review & editing

BÇ: Investigation, Formal analysis

All authors critically reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

This study was financially supported by Scientific Research Projects Coordinator (BAP) of Hatay Mustafa Kemal University (21.D.028).

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

The authors did not use any artificial intelligence technology in the writing of this article or in the creation of the images, tables, or graphs.

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Acta Nat. Sci. (2026) 7(1): 96-104
DOI: 10.61326/actanatsci.v7i1.404
e-ISSN: 2718-0638

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ACTA NATURA ET SCIENTIA PRENSIP

A Retrospective Long-Term Analysis of COVID-19 Cases Trends and a Predictive ARIMA Model Through Parameters Screening

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Please cite this paper as follows:
Essam Eissa, M. (2026). A Retrospective Long-Term Analysis of COVID-19 Cases Trends and a Predictive ARIMA Model Through Parameters Screening. *Acta Natura et Scientia*, *7*(1), 96-104. https://doi.org/10.61326/actanatsci.v7i1.404

ARTICLE INFO

Article History
Received: 31.08.2025
Revised: 14.12.2025
Accepted: 20.12.2025
Available online: 27.06.2026

Keywords:
COVID-19
SARIMA
Time Series
Forecasting
ARIMA
Public Health

ABSTRACT

Pandemics are always a source of serious concerns due to the devastating consequences to the communities and nations. Attempts to understand and predict the behavior of outbreaks are challenging as they are largely unpredictable. This research article presents an analysis of weekly COVID-19 cases data for selected Eastern Mediterranean Region (EMRO) country Egypt from January 2020 to May 2024. The study identifies a robust and parsimonious seasonal autoregressive integrated moving average (SARIMA) model for forecasting future trends based on performing comprehensive screening and a comparative analysis of various models. The data reveals a progression of the pandemic in Egypt through multiple waves of varying intensity. Among the models tested, the SARIMA((3,1,0), (0,0,0)) model was identified as the most suitable, demonstrating a strong balance between model fit and parsimony. The model passed all key diagnostic checks, including the Ljung-Box test for residual autocorrelation, with a high p-value of 0.977 at lag 12. This model provides a statistically sound and reliable framework for understanding and predicting the dynamics of the pandemic in Egypt based on the provided dataset. The model's strength lies in its simplicity and effectiveness, making it a powerful tool for policymakers. Second, the study demonstrates the applicability of the Box-Jenkins methodology to real-world epidemiological data, providing a practical example for similar future studies. The comprehensive screening and comparative analysis of multiple models ensure that the chosen model is not merely a good fit, but the best-fitting and most parsimonious option among the candidates. Finally, the analysis underscores the importance of accurate and consistent data reporting for effective pandemic management and modeling.

INTRODUCTION

The COVID-19 pandemic, a global health crisis of unprecedented scale, has underscored the critical need for robust, data-driven strategies to combat infectious disease outbreaks (Eissa, 2025a). As an unpredictable and rapidly evolving phenomenon, the spread of the SARS-CoV-2 virus has presented significant challenges for public health authorities worldwide (Rashed & Eissa, 2020). Predictive modeling, therefore, emerges as an indispensable tool for anticipating disease trajectories, informing policy decisions, and optimizing the allocation of scarce medical resources. Time series analysis, in particular, offers a powerful framework for dissecting the patterns inherent in epidemiological data, including autoregressive behaviors, trends, and seasonality. The Seasonal Autoregressive Integrated Moving Average (SARIMA) model, a widely recognized method in time series forecasting, is particularly well-suited for this task due to its capacity to capture both the long-term trends and cyclical fluctuations often observed in pandemic data (Tomov et al., 2023). Importantly, the application of the Box-Jenkins methodology is reinforced by the importance of the comprehensive screening and comparative analysis of multiple models, a rigorous process considered a best practice across various time series forecasting challenges (Yegin & Karcioglu, 2025a).

While numerous studies have applied time series models to COVID-19 data across different regions, country-specific analyses are crucial due to variations in local public health measures, population density, and social dynamics. For instance, the unique epidemiological landscape of the Middle East, with its specific demographics and healthcare systems, necessitates a tailored approach to forecasting (Ibrahim & Al-Said, 2023). While more complex non-linear models like artificial neural networks (ANNs) have been used for forecasting COVID-19 in Egypt (Saba & Elsheikh, 2020), the strength of the SARIMA model lies in its parsimony and interpretability, allowing policymakers to readily understand the epidemiological influence of the autoregressive components. This research addresses a gap in the literature by focusing exclusively on Egypt, a key country in the Eastern Mediterranean Region (EMRO), to provide a detailed, country-level analysis.

The primary objective of this study is to conduct a comprehensive analysis of weekly COVID-19 case data for Egypt from January 2020 to May 2024. Through a rigorous comparative analysis of multiple SARIMA models, this research aims to identify the most statistically sound and parsimonious model for forecasting future trends. The identified model will serve as a reliable tool for understanding the dynamics of the pandemic in the national context. The findings contribute to the broader body of epidemiological modeling research and provide a practical, evidence-based framework that can assist in future public health preparedness and response efforts.

MATERIALS AND METHODS

Data Acquisition and Preprocessing

Weekly COVID-19 cases for Egypt were acquired through filtering Excel sheet from publicly accessible dashboards maintained by the Humanitarian Data Exchange (Humdata) (Anonymous, 2026). The dataset spans from January 5, 2020, to May 26, 2024. The raw data included fields such as Date_reported, New_cases, Cumulative_cases, New_deaths, and Cumulative_deaths. Only cumulative cases were covered herein the scope of the current study.

For this time-series analysis, the primary variable of interest was the logarithm of cumulative cases (Log C.C.). This transformation was applied to stabilize the variance and linearize the growth trend observed in the raw cumulative case counts, which is a common practice in epidemiological modeling to meet the assumptions of linear models (Hyndman & Athanasopoulos, 2018).

Software

All data manipulation, time series analysis, and model fitting were performed using Minitab statistical software (Djauhari et al., 2020; Eissa, 2025b). Minitab offers robust tools for identifying, estimating, and validating Seasonal Autoregressive Integrated Moving Average (SARIMA) models.

SARIMA Model Identification and Estimation

The study followed the iterative Box-Jenkins methodology for time series modeling, which comprises identification, estimation, and diagnostic checking (Box & Jenkins, 1976). Experimentation with seasonality changes at 26 and 52 were investigated at the best model found at seasonality of 12.

Model Identification:

The initial step involved assessing the stationarity of the Log C.C. series. Visual inspection of the time series plot was subjected to investigation to reveal any trend, indicating non-stationarity. To achieve stationarity, first-order non-seasonal differencing (d=1) was applied. In case of the absence of a clear, strong seasonal pattern in the differenced series, as well as the context of the pandemic's global nature not necessarily following strict annual cycles, led to an exploration of models without seasonal differencing (D=0).

Preliminary orders for the non-seasonal autoregressive (p) and moving average (q) components, as well as seasonal autoregressive (P) and moving average (Q) components, were determined by examining the autocorrelation function (ACF) and partial autocorrelation function (PACF) plots of the differenced series. The seasonal period (s) was set to 12, corresponding to a roughly quarterly or multi-weekly cycle, which is a common consideration for health data.

Based on the ACF and PACF plots, several candidate SARIMA models with various combinations of (p, d, q), (P, D, Q)'s parameters were hypothesized and generated. A SARIMA model is often expressed as SARIMA ((p, d, q), (P, D, Q)), where lowercase letters indicate the non-seasonal component of the time series and uppercase letters indicate the seasonal component. Vector Autoregressive Models (or VAR Models) are used for multivariate time series.

Model Estimation and Selection

The methodology described by Eissa (2025b) has been followed for model estimation and selection.

- Each candidate SARIMA model was estimated through comprehensive screening using Minitab at p, q, P and Q from 0 to 5.
- Model selection was primarily guided by the Akaike Information Criterion corrected (AICc) and the Bayesian Information Criterion (BIC). Models with lower AICc and BIC values are generally preferred as they indicate a better balance between model fit and complexity (Hyndman & Athanasopoulos, 2018).
- The significance of individual parameters within each model was also assessed, with a p-value threshold of 0.05. Models containing insignificant parameters were generally excluded or modified to improve parsimony.

Diagnostic Checking

After model estimation, a crucial phase involved diagnostic checking to ensure the chosen model adequately represented the underlying data structure and that its residuals behaved as white noise (Eissa, 2025b).

Ljung-Box Test

The primary diagnostic tool used was the Ljung-Box Q statistic. This test assesses whether the autocorrelations of the residuals are significantly different from zero for a series of specified lags. A non-significant p-value ($p > 0.05$) for the Ljung-Box test indicates that the residuals are independently distributed (i.e., white noise), confirming that the model has captured all the systematic information in the time series (Ljung & Box, 1978). The test was performed on 12, 24, 36 and 48 lags.

Residual Plots

Visual inspection of the ACF of residuals and PACF of residuals plots was performed to corroborate the Ljung-Box test results. The absence of significant spikes outside the confidence limits in these plots further confirmed that the residuals were uncorrelated, indicating a well-fitted model.

Stability of Forecasts

The Time Series Plot with forecasts and their 95% confidence limits were also examined. Models exhibiting unstable forecasts or rapidly exploding confidence intervals were considered problematic, often indicating issues like over-differencing or parameter instability.

The model that demonstrated the lowest AICc and BIC among those with statistically significant parameters and residuals resembling white noise (as confirmed by the Ljung-Box test and residual plots) was selected as the final, recommended model.

RESULTS

The analysis of weekly COVID-19 data for Egypt revealed a dynamic progression of the pandemic from January 2020 to May 2024 in Figure 1. Time-series graph shows that growth occurs in steps of fast rates till reaching an almost plateau stabilization phase reaching 516023 during the study period. A comprehensive screening of various Seasonal Autoregressive Integrated Moving Average (SARIMA) models was conducted to identify the best-fitting and most parsimonious model for forecasting the logarithm of cumulative cases. Table 1 presents a ranked summary of the evaluated models based on their goodness-of-fit statistics, diagnostic checks, and parameter significance.

The recommended model is the SARIMA ((3,1,0), (0,0,0)) model. Mathematically, the expression of model can be illustrated by equation. The equation for this model is (Eq. 1):

$$(1-\phi_1 L-\phi_2 L^2-\phi_3 L^3)(1-L)\,Y_t=\epsilon_t \quad (1)$$

This can be expanded into the following form, which shows the relationship between the current value and previous values of the time series (Eq. 2):

where the explanation of terms: Y_t: The value of the time series at the current time, t, L: The Lag operator, where $L_k Y_t = Y_{t-k}$. For example, LY_t is the value of the series at the previous time, $t-1$, ϕ_1, ϕ_2, ϕ_3: The autoregressive parameters of the model. These coefficients, which were found to be highly significant in the analysis, determine the influence of the previous three differenced values on the current value, ϵ_t: The white noise error term at time t. This represents the random, unpredictable part of the time series that the model cannot explain and $(1-L)Y_t$: The first-order differencing of the time series. This term is crucial for making the data stationary by removing the non-constant trend of the cumulative cases.

The SARIMA ((3,1,0), (0,0,0)) model was selected as the most suitable. Despite some models having a lower AICc, they were deemed invalid as they failed the Ljung-Box test for residual autocorrelation. This diagnostic check is crucial for verifying that a model's residuals are random, or "white noise," which indicates that all the relevant patterns in the data have been captured. The recommended model had a high Ljung-Box p-value of 0.977, a strong indicator of a good fit. Furthermore, its core autoregressive (AR) terms were highly significant ($p \leq 0.002$), confirming their importance to the model's predictive power.

The diagnostic plots of the residuals also support the model's validity (Figures 2 and 3). Both the ACF of residuals and PACF of residuals plots show that the autocorrelations and partial autocorrelations are within the 5% significance limits, confirming that the residuals are uncorrelated and the model has effectively captured the data's patterns. The Time Series Plot for Log C.C. illustrates the historical data and the model's forecast for future values, along with a 95% confidence interval (Figure 4). As is typical with time series forecasts, the confidence interval widens over time, reflecting the increasing uncertainty of the predictions further into the future.

$$Y_t = Y_{t-1} + \phi_1(Y_{t-1} - Y_{t-2}) + \phi_2(Y_{t-2} - Y_{t-3}) + \phi_3(Y_{t-3} - Y_{t-4}) + \epsilon_t \quad (2)$$

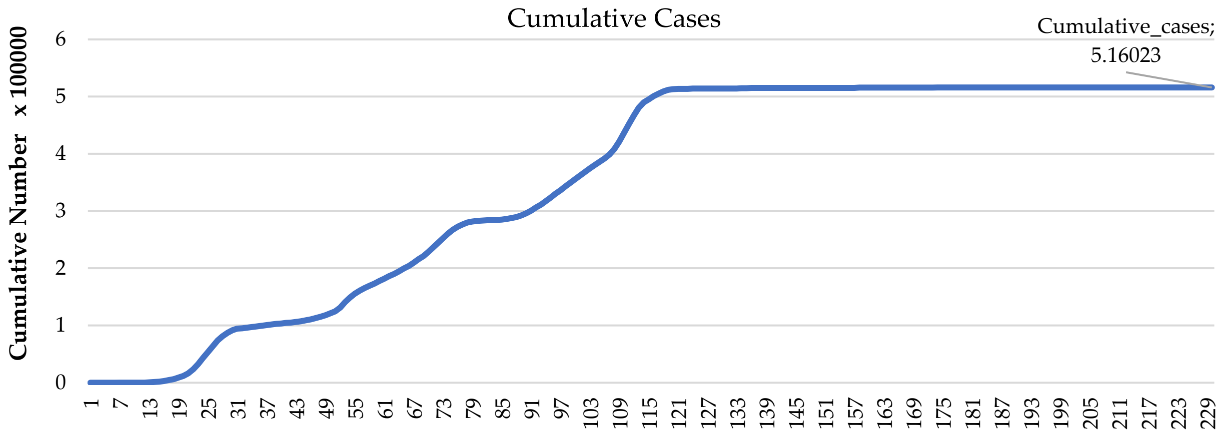

Figure 1. Time-series plot. Cumulative weekly cases from January 2020 to May 2024.

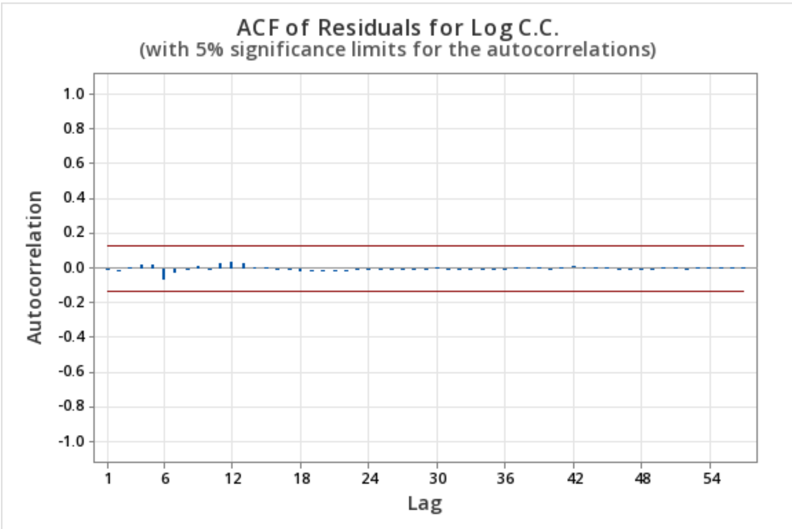

Figure 2. ACF of Residuals for Log C.C. This plot displays the autocorrelation function of the model's residuals, with the horizontal red lines representing the 5% significance limits. The plot confirms that the residuals are not correlated, indicating a well-fitted model.

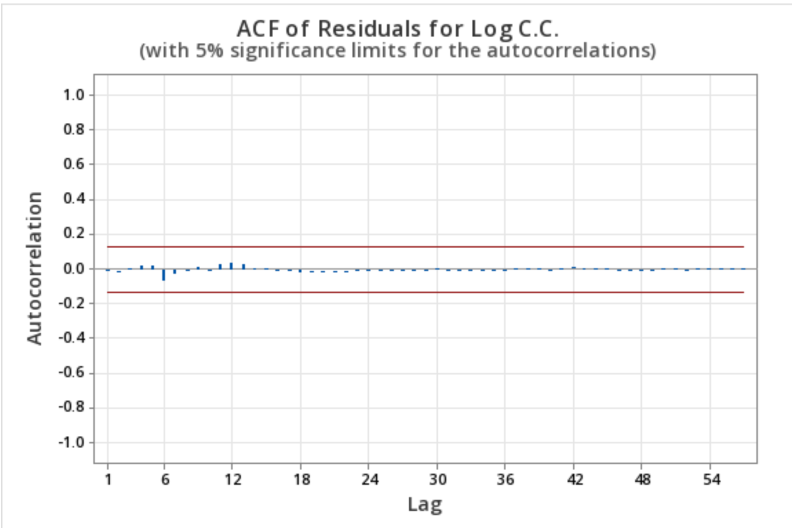

Figure 3. PACF of Residuals for Log C.C. This plot shows the partial autocorrelation function of the residuals, also with 5% significance limits. Like the ACF plot, the lack of significant spikes confirms that the model has captured all the relevant patterns in the data.

Table 1. Ranked SARIMA models for COVID-19 case data in Egypt in descending order of goodness-to-fit

Rank (by AICc)	Model Specification (p,d,q), (P,D,Q) s	Seasonal Period (s)	AICc	BIC	MS**	Ljung-Box P-Value (Lag 12)	Parameter Significance	Validity Assessment & Key Issues
1	SARIMA ((1,1,1)(3,0,2))	26	-786.042	-755.960	0.005189	0.029	SMA 52 and Constant are insignificant (p>0.24).	INVALID. Fails the Ljung-Box test for residual autocorrelation, indicating the model is unfit.
2	SARIMA ((5,1,1), (1,0,1))	52	-446.788	-413.460	0.0082105	0.613 (Pass)	Heavily over-parameterized; 4 of 9 parameters are insignificant.	INVALID. Despite a low AICc, the model is unnecessarily complex and not robust due to multiple insignificant parameters.
3	**SARIMA (3,1,0), (0,0,0))**	**12**	**-439.445**	**-422.545**	**0.0083368**	**0.977 (Pass)**	**Core AR terms are highly significant** (p≤0.002).	**VALID & RECOMMENDED. Best balance of fit (lowest AICc/BIC among valid models) and parsimony. Passes all diagnostic checks.**
4	SARIMA ((4,2,4), (2,0,0))	12	-435.236	-398.735	0.0072546	0	AR 4 and SAR 24 are insignificant (p>0.17).	INVALID. Fails the Ljung-Box test for residual autocorrelation with a highly significant p-value.
5	SARIMA ((2,1,2), (0,1,0))	12	-417.383	-400.768	0.0080575	0.277 (Pass)	All parameters are highly significant (p=0.000).	VALID. A strong and statistically sound model, but its AICc and BIC are notably higher than the recommended model.
6	SARIMA ((4,0,0),(0,1,1))	12	-414.339	-391.181	0.0073067	0.001	AR 2, AR 3, and Constant are insignificant.	INVALID. Over-parameterized with multiple insignificant parameters and fails the Ljung-Box test.
6	SARIMA ((2,0,3), (0,2,0))	12	-391.241	-371.696	0.0077561	0.03	MA 2 is insignificant (p=0.078).	INVALID. Fails the Ljung-Box test for residual autocorrelation.
7	SARIMA ((4,2,0), (2,1,1))	12	-405.627	-379.321	0.0088819	0.181 (Pass)	AR 3, AR 4, SAR 12, SAR 24, and SMA 12 are insignificant.	INVALID. Over-parameterized with multiple insignificant parameters
8	SARIMA ((2,2,0), (1,2,1))	12	-376.464	-360.177	0.0094143	0.732 (Pass)	Over-parameterized; SAR 12 and SMA 12 are highly insignificant (p>0.57).	INVALID. Unstable due to over-differencing and insignificant seasonal parameters, resulting in exploding forecast confidence intervals.
9	SARIMA (3,1,3), (0,2,0))	12	-391.232	-368.539	0.0077544	0.005	All parameters are statistically significant.	INVALID. Fails the Ljung-Box test for residual autocorrelation.

Note: *Focus on Lag 12 because all other Lags (24, 36 and 48) > 0.05 passing Modified Box-Pierce (Ljung-Box) Chi-Square Statistic. ** MS = variance of the white noise series.

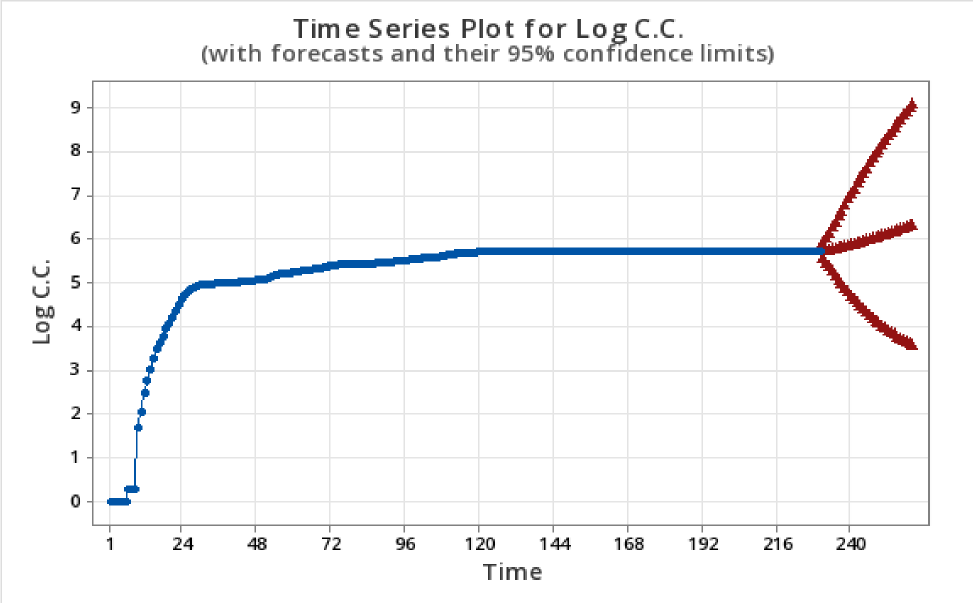

Figure 4. Time Series Plot for Log C.C. This plot shows the historical log-transformed cumulative case data (blue line) and the model's forecast for future values (red line) with a 95% confidence interval. The widening confidence interval reflects the increasing uncertainty of the forecast over time.

DISCUSSION

The selection of the SARIMA ((3,1,0), (0,0,0)) model as the best fit for the data was selected based on the principles of parsimony and statistical rigor in time series analysis. While the SARIMA (1,1,1), $(3,0,2)_{26}$ model had a lower AICc, its failure to pass the Ljung-Box test invalidates it as a reliable model. As noted by Box & Jenkins (1976), a model's residuals must be white noise for it to be considered a valid representation of the time series. The high p-value of 0.977 for the recommended model confirms that its residuals are indeed random, indicating that the model has successfully accounted for the data's underlying structure even though it demonstrated higher AICc.

The significance of the AR(3) component suggests that the current state of the pandemic (log-transformed cumulative cases) in Egypt is strongly influenced by its status from the previous three weeks, highlighting the autoregressive nature of disease spread. On the other hand, the first-order differencing (I(1)) is a critical component that stabilized the data by removing the non-constant trend of cumulative cases, which is a necessary step for applying ARIMA models (Pankratz, 1983). The absence of a seasonal component (0,0,0) in the final model is a notable finding. This suggests that while the pandemic experienced multiple waves, these cycles were not necessarily periodic over the 12-week seasonal period tested, making a simpler non-seasonal model the most appropriate fit. Future research could explore advanced, non-linear alternatives to the Box-Jenkins methodology, such as hybrid deep learning models optimized with Bayesian Optimization, which have demonstrated utility in complex time series forecasting for environmental systems (Yegin & Karcioglu, 2025b).

While the model is highly accurate for short-term forecasts, its predictive power diminishes over longer periods as new, unpredictable information (the white noise error term, ϵ_t) accumulates and compounds, leading to a wider range of possible outcomes. This is a fundamental property of all-time series forecasting models and does not, in this case, indicate issues like over-differencing or parameter instability, which would cause the forecast itself to become unstable or "explode" (e.g., producing wildly erratic or nonsensical values). However, the analysis is not without limitations, such as converting back the forecast output results to obtain comprehendible results. The data for new cases to be reported after May 2024, which affects the ability to capture recent trends accurately. This underscores the importance of continuous and reliable data collection for effective epidemiological modeling and public health decision-making (WHO, 2021). Despite this, the chosen model provides a statistically sound and valuable framework for understanding the pandemic's trajectory in Egypt, demonstrating the usefulness of the Box-Jenkins methodology in a real-world public health context and overcoming previous modelling attempts limitations (Eissa, 2022, 2023). It should be noted that accuracy of modelling and prediction is largely dependent on the quality of the original raw data which must be ensured to be a true representation of the real-world outbreak situation.

CONCLUSION

Based on the analysis performed, the most effective and statistically sound model for forecasting the logarithm of cumulative COVID-19 cases nationwide is the SARIMA ((3,1,0), (0,0,0) model. This model was chosen for its optimal balance between a strong statistical fit and parsimony, a fundamental principle in time series modeling. The model's validity is confirmed by its successful performance on key diagnostic checks, including a high p-value of 0.977 at lag 12 and 1.000 at 24, 36 and 48 lags in the Ljung-Box test for residual autocorrelation. This result indicates that the model's residuals are random and uncorrelated, confirming that the model has successfully captured the underlying patterns of the time series. The study's significance lies in its ability to provide a robust and reliable framework for understanding and modelling the dynamics of the pandemic in particular geographical region. The identified model serves as a practical, evidence-based tool for policymakers and public health officials, offering valuable insights that can inform future preparedness and response strategies. The analysis also underscores the critical need for accurate and consistent public health data reporting for effective epidemiological modeling.

Compliance with Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Funding

Not applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure

Generative AI was used for grammatical, writing and assistant supportive tool and review throughout the manuscript. The author validated all outputs and assumes full responsibility for the content.

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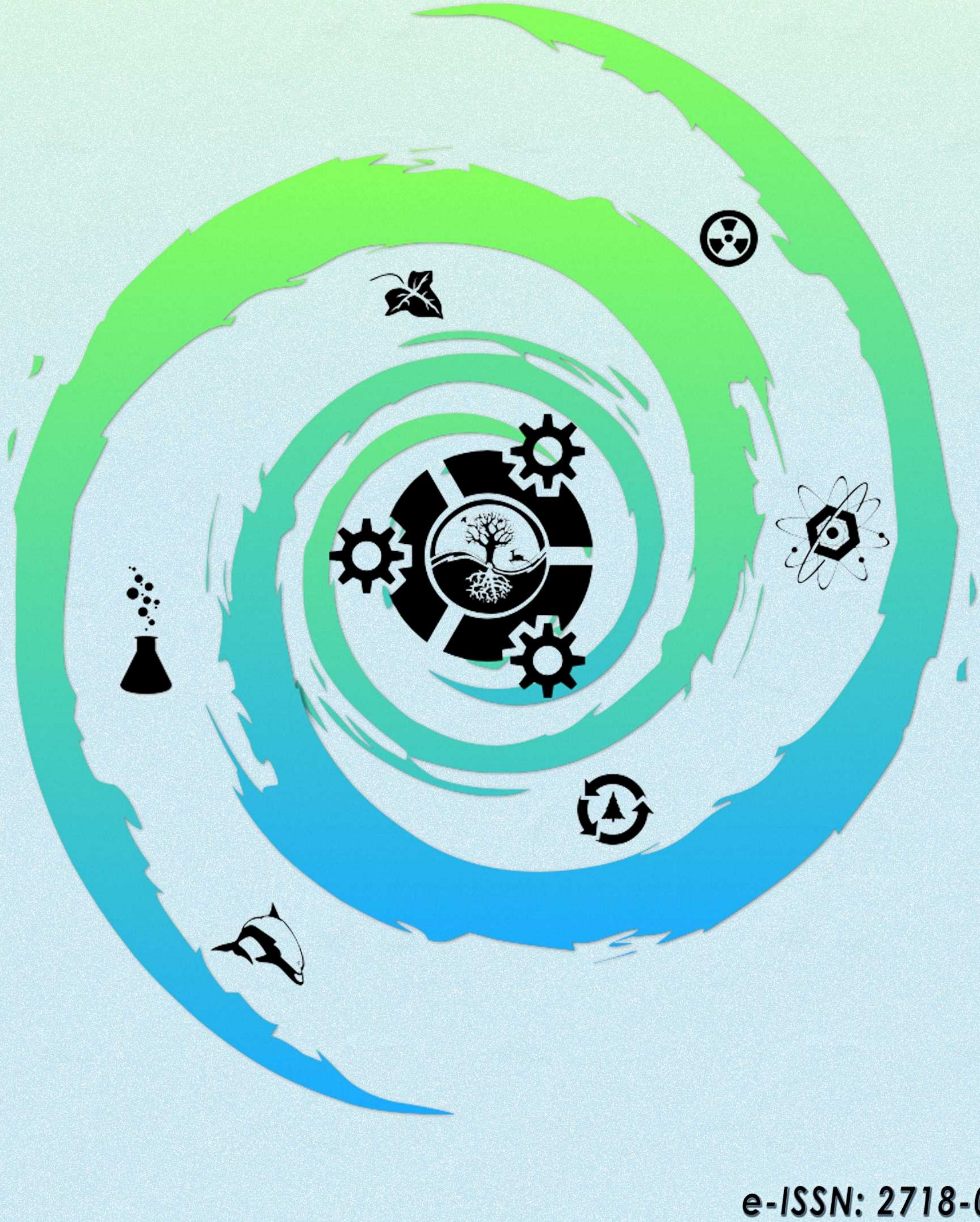

e-ISSN: 2718-0638

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