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EFFECT OF POLYPHENOLS RICH SEED COATING ON ENHANCEMENT OF SEEDLING PHYSIOLOGY AND ANTIOXIDANT ACTIVITY OF MAIZE (*ZEA MAYS* L.) SEEDS

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ABSTRACT
The use of plant derived bioactive compounds as eco-friendly seed enhancement agents has gained considerable attention in sustainable agriculture. The present study aimed to develop and characterize a polyphenol rich seed coating formulation based on *Terminalia chebula* pericarp powder to evaluate its effect on seed germination and seedling vigour of maize (*Zea mays* L.). The formulation was characterized using GC–MS, FTIR and XRD analyses. GC–MS analysis identified 1,2,3-benzenetriol (Pyrogallol) as the major constituent (46.55%), followed by bisphenol (23.67%) and phenol (2.22%) indicating a high concentration of bioactive phenolic compounds. FTIR analysis confirmed the presence of hydroxyl, carbonyl, aliphatic, aromatic, and carbohydrate functional groups. XRD analysis demonstrated a predominantly amorphous structure with minor crystalline regions. The efficacy of the formulation was evaluated through seed coating treatments in maize. Among the treatments, T_4 at 100 g kg^{-1} seed consistently recorded the highest germination percentage (97%), speed of germination (5.8), root length (24.5 cm), shoot length (44.9 cm), dry matter production (3.36 g), Vigour Index I (6732) and Vigour Index II (326) compared to the untreated control. Antioxidant enzyme activity also higher in treated seeds. The enhanced germination and seedling growth were attributed to the antioxidant and growth promoting effects of polyphenolic compounds present in the formulation. The study concludes that the polyphenol rich *Terminalia chebula* pericarp powder based seed coating formulation effectively improves maize seed germination, seedling vigour and early plant growth. The formulation offers a promising, environmentally friendly alternative to conventional synthetic seed treatments and has potential applications in sustainable crop production.

KEYWORDS: *Terminalia chebula*, polyphenols, seed germination, seedling vigour, GC–MS, FTIR, XRD.

INTRODUCTION
Maize (*Zea mays* L.) is one of the most important cereal crops worldwide and serves as a staple food, livestock feed and industrial raw material. The productivity of maize is greatly influenced by seed quality parameters like germination potential and seedling vigour, particularly during the early stages of crop establishment [1]. Rapid and uniform germination coupled with vigorous seedling growth is essential for achieving optimum plant population and higher crop productivity [2]. However, various biotic and abiotic stresses often impair seed germination and seedling development resulting in reduced crop performance. Therefore, the development of sustainable seed enhancement technologies has become increasingly important in modern agriculture [3].
Seed coating has emerged as an effective strategy to enhance seed germination, seedling vigour and overall crop resilience [4]. By improving seed performance, seed coating offers significant benefits for large scale farming, precision agriculture and organic crop production. Additionally, seed coatings contribute to reducing seed borne diseases and ensure uniform crop establishment under varying climatic conditions [5]. In recent years, seed coating has emerged as an innovative and effective strategy to enhance seed germination, improve seedling vigour and strengthen crop resilience against biotic and abiotic stresses. Advanced seed coating technologies incorporate essential nutrients, microbial inoculants, bio-stimulants, and protective agents that not only boost initial plant growth but also offer long term benefits such as disease resistance, drought tolerance and enhanced nutrient uptake [6]. By ensuring uniform seed emergence and early stage development, seed coatings contribute to increased crop uniformity, higher yields and improved resource efficiency in agricultural production systems [7].
Polyphenols are a diverse group of naturally occurring secondary metabolites widely distributed in plants. These compounds possess strong antioxidant, antimicrobial and growth regulating properties making them valuable candidates for agricultural applications. Polyphenols can protect germinating seeds against oxidative stress by scavenging reactive oxygen species (ROS), enhance nutrient mobilization, stimulate enzymatic activities and improve seedling growth and vigour [8,9]. In addition, phenolic compounds have been reported to suppress seed

borne pathogens and improve plant defense mechanisms, thereby contributing to healthier crop establishment [10].

Terminalia chebula Retz., commonly known as haritaki, belongs to the family Combretaceae and is widely recognized as a medicinal plant rich in bioactive phytochemicals. The pericarp of *Terminalia chebula* contains rich polyphenolic compounds which exhibit remarkable antioxidant, antimicrobial, anti-inflammatory and free radical scavenging activities [11]. Due to these biological properties, *Terminalia chebula* has been extensively investigated in pharmaceutical and nutraceutical applications. However, limited information is available regarding its potential utilization as a polyphenol rich herbal seed coating agent for improving seed germination and seedling vigour in agricultural crops.

Therefore, the present study was undertaken to develop and characterize a polyphenol rich herbal *Terminalia chebula* pericarp powder based seed coating formulation to evaluate its effect on seed germination and seedling vigour of maize. Also, to characterize the formulation using GC–MS, FTIR and XRD techniques and to assess its potential as an eco-friendly seed enhancement technology for improving maize seed performance and early crop establishment.

MATERIALS AND METHODS

Experimental site and plant materials

This study was carried out during 2025–2026 at the Department of seed science and technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Genetically pure seeds of Maize COH(M) 11 used for the study have been procured from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore.

Polyphenols source

Commercially available *Terminalia chebula* pericarp powder used as a polyphenols source for this study.

Identification of polyphenols through GCMS (Gas Chromatography Mass Spectroscopy) analysis

The polyphenol source was analysed to identify its polyphenol content using GC-MS instrument.0.3g of *Terminalia chebula* pericarp powder was transferred into 2ml tubes then it add with 1.4 ml of 100% methanol and vortexed. Then Add 50 µl of ribitol (0.2mg/ml water) and incubate at 70°C with continuous shaking for 15 minutes. Then its Centrifuged at 12000 rpm for 20 minutes at 4°C. Supernatant was transferred to 0.2 µm filter and add 1.4ml of water and 750 µl chloroform was mixed and centrifuged at 12000 rpm for 10 min. Then 1 ml of Upper polar phase was transferred to new tubes and remaining 1 ml of supernatant was transferred into another new tube for future use stored at -80°C**.**Concentrate of upper phase tubes using concentrator at 45°C for 3 hrs. After that,50 µl of methoxamine hydrochloride (20mg /ml of pyridine) was added to the tried fraction and tubes were incubated at 37 °C for 2hours with continuous shaking. Then add 80 µl MSTFA was added and again incubated at 37°C for 30 minutes. Then its centrifuged at 10000 rpm for 3 min and the supernatant transferred into GCMS vials. Then it was s analysed through GCMS instrument to identify the polyphenols compounds.

Development of polyphenols rich seed coating formulation

A new polyphenols rich formulation was developed in this study. The polyphenols rich *Terminalia chebula* pericarp powder used as an active ingredient (ai). It was initially added with linseed oil to make slow release of the active ingredients. Initially the stock solution (brown colour pigment, polymer (Incotec Pvt. Ltd, India), pectin, gelatin, carboxy methyl cellulose, kaolin, potassium silicate mixed for 1hr 30 minutes with 400 RPM at magnetic stirrer) 10 gram was prepared. This stock solution act as surfactant for this formulation. Then the active ingredient was added with different concentrations (5g, 5.5g, 6g and 6.5g ai) on stock solution(10g) and it was thoroughly mixed for 30 minutes with 1% of Aazdirachtin (3ml) at magnetic stirrer with 400 RPM. The above formulation was prepared for base for this study. It has converted to 100g of formulation for coating the one kilogram of seeds. The formulation coated with different treatments *viz.,* T_0- Control (Uncoated), T_1- Stock solution (No active ingredient) (100g/kg), T_2-100g/kg (5g ai), T_3-100g/kg (5.5g ai)), T_4-100g/kg (6g ai) and T_5-100g/kg (6.5g ai). Later the formulations were coated over the seeds at 100 g /kg of seeds followed by drying for about 15 min. Then the coated seeds were later subjected to assess its seedling vigour under germination room conditions.

Characterization of polyphenol based formulation

Polyphenols rich formulation were characterized through different instrumental methods.

Fourier transform infrared (FT-IR) spectroscopy: This method was used to determine the functional groups and chemical bonds of the constituents in the the polyphenol source formulation. Shimadzu FTIR spectrometer model was used, the sample was placed on the port and it was exposed to infrared radiation with a wavelength ranging from 4000 to 400 cm–1. The sample reacts with the radiation and absorb specific frequencies, based on its functional groups. The absorption pattern is a unique fingerprint of the functional groups and chemical bonds present in the sample (polyphenol source). Then, it was detected using detector between 4000 to 400 cm–1.

X-ray diffraction (XRD) analysis: X-ray diffraction (XRD) analysis was done to know the structural makeup, morphology and crystallographic characteristics of the samples. The analysis was performed using a Bruker D2-Phase equipped with Cu Kα monochromatic radiation (ʎ= 1.5406 Å). The instrument was operated at a voltage of 45 Kv and 30 mA and the scans were performed over a °2Theta range of 10° to 90° in continuous scan mode. The resulting diffraction data was collected and plotted using ORIGIN Ver.8.5 software.

Physiological study

The coated seeds were sown under the germination room conditions to assess its physiological parameters.

The physiological parameters were taken for 14 DAS (Days After Sowing). The physiological parameters are given below

Speed of emergence: According to [12] Daily counts of germinated seeds were recorded until the final count day (7th day) and the speed of germination was calculated using the formula given below,

$$\text{Speed of germination} = \frac{X1}{Y} + \frac{X2 - X1}{Y2} + \frac{Xn - Xn - 1}{Yn}$$

X_1- No. of seeds germinated during 1st day
X_2- No. of seeds germinated during 2nd day
X_n- No. of seeds germinated during nth day
Y_1- No. of days from sowing to the first count
Y_2- No. of days from sowing to the second count
Y_n- No. of days from sowing to the nth count

Germination (%): Germination percentage was assessed under raised bed conditions in shade net. The seeds were placed on sandy loam soil for 4 replications was put for each treatment. As per the guideline of [13], the final germination percentage was computed on 7th day after sowing using the formula given below

$$\text{Germination \%} = \frac{\text{No. of normal seedlings}}{\text{Total no. of seeds sown}} \times 100$$

Root length (cm): For root length measurement of four replications was measured on 14 DAS. From the germination test, ten healthy seedlings were randomly selected and their root lengths were measured from the collar region to the root tip using a measuring scale. The average mean values were expressed in centimeter.

Shoot length (cm): The seedlings used for root length measurement were also utilized to measure shoot length. Shoot length was measured from the collar region to the tip of the primary leaf using a measuring scale and the calculated mean value was expressed in centimeter.

Dry matter production (g 10 seedlings^{-1}): Ten seedlings used for seedling length measurement were placed in a brown paper cover and shade dried for 24 hours. Then, it was oven-dried at 80°C for 24 hours and cooled in a desiccator for 30 minutes. The dry weight was measured using an electronic balance and the average weight was expressed as g 10 seedlings^{-1}.

Vigour Index: According to [14] the vigour index of the seedlings was computed using the below given formula and expressed in whole number.

Vigour index I = Germination (%) x Total seedling length (cm).

Vigour index II = Germination (%) x Dry matter production (g10 seedlings^{-1})

Stem Anatomical Analysis: Stem samples were collected from 14 days old maize seedlings from control and coated seeds. Transverse sections (TS) of the stem were prepared manually using a sharp razor blade. The sections were stained with 1% safranin and mounted on microscopic slide with the help of cover slip. Microscopic observations were carried out using a compound light microscope at 40× magnification. Images were captured and analysed to assess anatomical variations in epidermis, hypodermis, ground tissue, vascular bundles, xylem and phloem among control and coated seedlings.

Biochemical study

To find out the antioxidant enzymes present in the untreated and treated seeds the below antioxidant activity test was conducted.

Antioxidant enzymes activity: Antioxidant enzymes such as catalase and peroxidase were estimated with both treated and untreated seeds. The catalase enzyme activity was calculated and expressed in µg H_2O_2 mg^{-1} min^{-1} [15], which depends on the amount of catalase that breaks down H_2O_2 into water. Peroxidase activity was expressed in ΔOD 430 mg^{-1} min^{-1} [16].

Statistical analysis. The experiment was carried out using Completely Randomized Design (CRD) with four replications. Experiment's data were statistically analysed using AGRES software. The treatment means were compared by Least Significant Differences (17). The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance. If the F test is non-significant, it was denoted as non-significant.

RESULTS

Identification of polyphenols using GCMS analysis

GC–MS analysis of the *Terminalia chebula* pericarp powder identified three major compounds based on their retention times and peak area percentages (Table 1) (Figure 1). Among the identified constituents, 1,2,3-benzenetriol (Pyrogallol) was the predominant compound, accounting for 46.55% of the total peak area, followed by bisphenol (23.67%) and phenol (2. 22%).The high abundance of 1,2,3-benzenetriol indicates that the extract is rich in polyphenolic constituents. This compound possesses strong antioxidant activity due to its three hydroxyl groups, which can scavenge free radicals and protect seed tissues from oxidative damage. Bisphenol represented the second most abundant compound (23.67%). In polymeric seed coatings, bisphenol related compounds may contribute to coating strength and stability. Nevertheless, their potential to interfere with plant hormone signaling

suggests that their concentration should be carefully considered to avoid adverse effects on root and shoot development. Phenol, detected at 2.22%, was present in comparatively low abundance. At low concentrations, phenolic compounds can enhance plant defense responses by stimulating defense related enzymes and suppressing pathogen growth. However, higher concentrations may become phytotoxic and inhibit root elongation and cell division.

Characterization of polyphenols rich formulation

The FTIR spectrum exhibited a broad absorption band at 3357.70 cm^{-1} corresponding to O–H stretching vibrations of hydroxyl groups, indicating the presence of hydrogen bonded alcohols and phenolic compounds. The absorption bands at 2925.06 and 2851.31 cm^{-1} were assigned to asymmetric and symmetric stretching vibrations of aliphatic C–H bonds. A prominent band at 1774.60 cm^{-1} indicated carbonyl (C=O) stretching, suggesting the presence of ester or ketone functional groups. The band at 1640.56 cm^{-1} was attributed to aromatic C=C stretching or absorbed water. The peak at 1454.18 cm^{-1} corresponded to CH_2 bending vibrations, while the strong absorptions at 1159.20, 1105.79, and 1032.42 cm^{-1} were assigned to C–O and C–O–C stretching vibrations characteristic of polysaccharides such as cellulose and hemicellulose. Overall, the FTIR analysis confirmed the presence of hydroxyl, aliphatic, carbonyl, and carbohydrate functional groups in the sample (Fig. 2a)

The XRD analysis pattern exhibits a broad diffraction halo centred at approximately 20–23° (2θ), indicating that the sample is predominantly amorphous in nature. A sharp diffraction peak observed near 28–29° (2θ) confirms the presence of a minor crystalline phase embedded within the amorphous matrix. The weak reflections at higher diffraction angles further suggest limited crystallinity. Overall, the XRD results demonstrate that the sample possesses a semi-crystalline structure with a dominant amorphous character (Fig. 2b).

Physiological parameters

The effect of polyphenols rich seed coating formulation on seed germination and seedling vigour of maize was observed on 14th day after sowing (DAS). The result revealed that, T_4-100g/kg (5.8) followed by T_3 - 100 g/kg (5.7) recorded higher speed germination rate than T_0 - Control (5.4). Germination percentage recorded was higher in T_4-100 g/kg (97 %) followed by T_3 - 100 g/kg (95 %) and lower germination percentage was observed in T_0 - Control (93 %). Root length recorded was higher in T_4-100 g/kg (24.5 cm) followed by T_3 - 100 g/kg (23.1 cm) and lower in T_0 - Control (20.4 cm). Shoot length recorded was higher in T_4-100 g/kg (44.9 cm) followed by T_3 - 100 g/kg (43.5 cm) and lower in T_0 - Control (39.9 cm). Dry matter production recorded was higher in T_4 - 100 g/kg (3.36 g) followed by T_3-100 g/kg (2.99 g) and lower in T_0 - Control (2.69 g). Vigour Index I recorded was higher in T_4-100g/kg (6732) followed by T_3 - 100 g/kg (6327) and lower in T_0 - Control (5608). Vigour Index II recorded was higher in T_4-100g/kg (326) followed by T_3 - 100 g/kg (284) and lower in T_0 - Control (250) Thus, the treatments T_4-100g/kg was the most effective treatment for all physiological parameters like germination (97 %), root length (24.5 cm), shoot length (44.9), dry matter (3.36 g), vigour index I (6732) and vigour index II (326) compared than other treatments (Table 1) (Fig.3).

Stem anatomical analysis of seedlings

The transverse section of the stem from control maize seedlings (14 DAS) showed a normal monocotyledonous stem anatomy characterized by a well-defined epidermis, sclerenchymatous hypodermis, parenchymatous ground tissue, and scattered vascular bundles. The vascular bundles exhibited typical collateral and closed organization with distinct xylem and phloem tissues. The metaxylem vessels were moderately developed, and no noticeable anatomical modifications or tissue proliferation were observed, indicating normal stem development under untreated conditions. The transverse section of treated maize seedlings (14 DAS) revealed improved anatomical development compared with the untreated control. The stem exhibited compactly arranged parenchymatous ground tissue and well-developed vascular bundles with prominent xylem and phloem differentiation. The xylem vessels appeared larger and more organized, indicating enhanced conducting capacity. Increased tissue compactness and vascular development in treated seedlings suggest that the polyphenol-based seed coating treatment positively influenced stem anatomical growth and seedling establishment(Fig.4)**.**

Antioxidant enzymes activity of seeds

Antioxidant enzymes activity like catalase and peroxidase activity were assessed for polyphenol rich formulation coated and uncoated seeds. For catalase activity, the result revealed that T_4-100g/kg (28.98 µg H_2O_2 mg^{-1} min^{-1}) followed by T_3 - 100 g/kg (28.65 µg H_2O_2 mg^{-1} min^{-1}) recorded higher catalase activity compared than T_0 - Control (28.27 µg H_2O_2 mg^{-1} min^{-1}). For Peroxidase activity, the result revealed that T_4-100g/kg (7.32 ΔOD 430 mg^{-1} min^{-1}) followed by T_3 - 100 g/kg (6.93 ΔOD 430 mg^{-1} min^{-1}) recorded higher catalase activity compared than T_0 - Control (5.85 ΔOD 430 mg^{-1} min^{-1}) (Fig.5).

DISCUSSION

The GC–MS analysis of *Terminalia chebula* pericarp powder revealed the presence of several phenolic compounds, with 1,2,3-benzenetriol (pyrogallol) as the dominant constituent, followed by bisphenol and phenol. The predominance of pyrogallol indicates that the extract is highly enriched with polyphenolic compounds, which

are well known for their antioxidant and antimicrobial properties. Similar observations have been reported for *Terminalia chebula*, where hydrolysable tannins and phenolic compounds contribute significantly to its biological activity [11,18]. Pyrogallol possesses strong free radical scavenging activity due to the presence of three hydroxyl groups on the aromatic ring, enabling it to donate hydrogen atoms and stabilize reactive oxygen species (ROS). This antioxidant potential may protect seeds from oxidative damage during germination and storage [19]. The second major compound identified was bisphenol. Bisphenol related compounds are aromatic phenolic that may contribute to the structural integrity and stability of polymer-based seed coatings because of their rigidity and hydrophobic characteristics. Their presence may therefore enhance coating adherence and durability on seed surfaces. Nevertheless, bisphenol derivatives are also known to interact with endocrine and signaling pathways in biological systems [20]. Phenol was detected at a comparatively low level. Phenolic compounds at low concentrations can play beneficial roles in plant defense by inducing defense related enzymes and inhibiting pathogenic microorganisms [8]. This may contribute to improved seed protection against fungal or bacterial infection. However, phenol and related compounds are also recognized for their allopathic and phytotoxic effects at higher concentrations, where they can inhibit cell division, reduce root growth and impair nutrient uptake [21].
The FTIR analysis revealed the presence of several important functional groups that are characteristic of phenolic compounds, carbohydrates and other bioactive constituents present in the sample. The broad absorption band observed at 3357.70 cm^{-1} was attributed to O–H stretching vibrations of hydroxyl groups, indicating the presence of hydrogen bonded alcohols and phenolic compounds. Hydroxyl groups are commonly associated with polyphenols, tannins and flavonoids, which are known for their antioxidant and antimicrobial properties. In *Terminalia chebula*, phenolic compounds such as gallic acid, ellagic acid and pyrogallol have been reported as major bioactive constituents. Its contributing significantly to its antioxidant activity [11]. The presence of a strong O–H stretching band therefore supports the GC–MS findings that identified polyphenolic compounds in the extract. The absorption bands at 2925.06 cm^{-1} and 2851.31 cm^{-1} correspond to asymmetric and symmetric stretching vibrations of aliphatic C–H bonds. These peaks indicate the presence of aliphatic chains derived from fatty acids, lipids and waxes or hydrocarbon components. Similar bands have been reported in plant extracts and seed oils containing long chain fatty acids and lipid derivatives [22]. In the present study, these peaks may also be associated with the linseed oil component of the formulation, which is rich in unsaturated fatty acids and contributes to the hydrophobic and film-forming properties of the seed coating. A prominent absorption band at 1774.60 cm^{-1} was assigned to carbonyl (C=O) stretching vibrations, suggesting the presence of ester, ketone, or carboxylic acid derivatives. Carbonyl-containing functional groups are commonly found in plant metabolites and polysaccharide-associated esters. The presence of this peak may indicate esterified phenolic compounds or lipid-derived constituents from linseed oil. Similar carbonyl absorption bands have been observed in plant-based formulations and natural polymers containing ester linkages [23]. Carbonyl groups are important because they can participate in intermolecular interactions and contribute to the stability of coating formulations. The band at 1640.56 cm^{-1} was attributed to aromatic C=C stretching vibrations and may also be associated with absorbed water molecules. This peak confirms the presence of aromatic ring containing compounds, particularly phenolic substances. Aromatic compounds are important contributors to the antioxidant and antimicrobial activities of medicinal plant extracts. Previous studies on *Terminalia chebula* have reported similar FTIR bands associated with tannins and phenolic acids, which are responsible for many of the plants biological activities [24]. The absorption peak at 1454.18 cm^{-1} corresponded to CH_2 bending vibrations, indicating the presence of methylene groups associated with aliphatic compounds and structural polysaccharides. Furthermore, the strong peaks observed at 1159.20, 1105.79, and 1032.42 cm^{-1} were assigned to C–O and C–O–C stretching vibrations, which are characteristic of polysaccharides such as cellulose and hemicellulose. These peaks indicate the presence of carbohydrate-based structures and glycosidic linkages within the sample. Similar FTIR signatures have been reported for cellulose rich plant materials and natural biopolymer formulations. The occurrence carbohydrate related functional groups may contribute to coating adhesion, film formation and moisture retention around the seed surface [25].
The X-ray diffraction (XRD) analysis revealed that the synthesized formulation possesses a predominantly amorphous structure, as evidenced by the broad diffraction halo centered between 20° and 23° (2θ). Broad diffraction peaks are characteristic of amorphous materials, where the constituent molecules lack long-range crystalline order. Such amorphous behavior is commonly observed in plant derived extracts, biopolymers, and organic formulations due to the complex arrangement of phenolic compounds, polysaccharides, and other biomolecules [26]. The predominance of the amorphous phase suggests that the bioactive constituents of *Terminalia chebula* and linseed oil are distributed in a disordered matrix rather than forming highly ordered crystal lattices. The presence of a distinct diffraction peak near 28–29° (2θ) indicates the existence of a minor crystalline phase within the predominantly amorphous matrix. This crystalline region may arise from naturally occurring mineral constituents, crystalline fractions of polysaccharides, or ordered domains formed during the formulation process. Similar observations have been reported in herbal and biopolymer based formulations where small crystalline regions coexist with amorphous organic components [27]. The coexistence of amorphous and crystalline phases suggests that the formulation possesses a semi-crystalline structure, combining the advantages of both structural forms. The weak diffraction reflections observed at higher diffraction angles further support the presence of limited crystallinity. These low-intensity peaks indicate that only a small proportion of the material contributes to crystalline ordering, while the majority remains amorphous. In natural polymeric systems, such as

cellulose-containing materials, weak crystalline reflections are often associated with ordered cellulose micro domains embedded within an amorphous matrix [28]. The low crystallinity observed in the present study may therefore be attributed to the heterogeneous composition of the formulation, which contains phenolic compounds, carbohydrates and lipid-derived constituents. From a functional perspective, the predominance of the amorphous phase may be beneficial for agricultural applications. Amorphous materials generally exhibit higher solubility, faster hydration, and greater bioavailability than highly crystalline materials because their molecules are less tightly packed and more accessible to the surrounding environment [29].

The improved germination and seedling growth observed in T_4-100g/kg may be attributed to the high concentration of polyphenolic compounds present in the formulation. Polyphenols are known to function as natural antioxidants that protect germinating seeds from oxidative stress by scavenging reactive oxygen species (ROS) generated during imbibition and early metabolic activation. During seed germination, controlled ROS production is necessary for breaking dormancy and stimulating cellular metabolism; however, excessive ROS accumulation can damage cellular membranes, proteins, and nucleic acids. The antioxidant activity of polyphenols helps maintain ROS balance, thereby improving germination performance and seedling growth [30,31]. The enhanced root and shoot growth recorded in the treated seeds may also be associated with the ability of polyphenols to regulate physiological and biochemical processes involved in plant development. Phenolic compounds can stimulate enzymatic activities related to reserve mobilization, resulting in more efficient utilization of stored nutrients during germination. Improved mobilization of carbohydrates, proteins, and lipids provides sufficient energy and metabolites for rapid root and shoot elongation. Similar findings were reported by [32], who observed that polyphenol rich plant extracts promoted seedling growth by enhancing antioxidant defense systems and metabolic activity. The higher dry matter production and vigour indices recorded in T_4-100g/kg treated seeds further indicate improved seedling health and biomass accumulation. Polyphenols have been reported to enhance nutrient uptake and protect seedlings against environmental stress conditions, thereby contributing to increased biomass production. In addition, the antimicrobial properties of phenolic compounds may reduce the incidence of seed-borne pathogens during germination, resulting in healthier seedlings and improved vigour [8]. The superior performance of T_4-100g/kg compared to lower concentrations suggests that the concentration of polyphenols was optimal for stimulating physiological processes without causing phytotoxic effects. Although phenolic compounds can inhibit growth at excessive concentrations, appropriate levels often act as bio stimulants that enhance seed germination, root development, and seedling establishment [33].

Stem anatomical analysis showed enhanced vascular differentiation observed in treated seedlings may be attributed to the bioactive polyphenolic compounds present in the seed coating formulation. Polyphenols are known to influence plant growth by modulating hormonal balance, stimulating cell division, and improving nutrient mobilization during early seedling establishment [34]. Improved xylem development increases water transport efficiency from roots to shoots, thereby supporting greater biomass accumulation and seedling vigour [35]. [36] stated that phenolic rich botanical extracts enhanced cellular organization and vascular tissue development in young seedlings. Improved vascular architecture has been associated with enhanced translocation of water, minerals, and photosynthetic, resulting in better growth performance under both normal and stress conditions [37]. Polyphenolic compounds also possess strong antioxidant properties that protect meristematic tissues from oxidative damage during germination and early growth stages. Enhanced antioxidant activity can maintain membrane integrity and support continuous cell division, ultimately leading to improved anatomical development [38]. Seed treatments enriched with natural phenolics increased lignification and vascular differentiation, contributing to stronger stem structures and improved seedling establishment [39].

Antioxidant enzymes activity like catalase activity was higher in T_4-100g/kg, while the untreated control recorded the lowest activity. Catalase is a key antioxidant enzyme that rapidly decomposes hydrogen peroxide into water and oxygen, thereby preventing oxidative damage to cellular components. The increased catalase activity observed in coated seeds suggests that the polyphenol-rich formulation enhanced the antioxidant defense system, enabling more efficient scavenging of ROS generated during germination. Similar increases in catalase activity following treatment with plant derived biostimulants and phenolic compounds have been reported by [40]. Likewise, peroxidase activity was markedly higher in the coated seed treatments compared than control. Peroxidase plays an important role in the detoxification of hydrogen peroxide and contributes to cell wall strengthening through lignification and phenolic cross-linking reactions [41].

CONCLUSION

The present study revealed that polyphenol rich seed coating formulation using *Terminalia chebula* pericarp powder for improving seedling physiology and antioxidant activity in maize. GC–MS analysis confirmed the presence of bioactive phenolic compounds, with 1,2,3-benzenetriol (Pyrogallol) as the major constituent, indicating a high polyphenol content with potential antioxidant and antimicrobial properties. FTIR analysis verified the presence of hydroxyl, carbonyl, aliphatic, aromatic, and carbohydrate functional groups, while. XRD analysis demonstrated a predominantly amorphous structure with minor crystalline regions, suggesting favourable properties for the controlled release and bioavailability of active compounds. The physiological parameters showed that the polyphenol rich seed coating significantly enhanced germination and seedling growth compared with the untreated control. Among the treatments, T_4-100g/kg seeds consistently recorded the highest speed of germination, germination percentage, root length, shoot length, dry matter production and vigour indices. Stem anatomical analysis also confirmed coated seeds showed improved anatomical structure, Antioxidant enzymes

activity also showed higher in treated seeds compared than control. The improved physiological performance can be attributed to the antioxidant activity of polyphenols, which protect germinating seeds from oxidative damage, enhance metabolic processes, improve nutrient mobilization and support healthy seedling development. The antimicrobial properties of phenolic compounds may also have contributed to improved seedling establishment by reducing microbial interference during germination. Therefore, polyphenol-based seed coating technology has considerable potential as a sustainable alternative to conventional synthetic seed treatments and may contribute to improved crop establishment and agricultural productivity.

AUTHOR CONTRIBUTION

First author (Arulmoorthy K) executed the research work, article writing and uploading; Second author (Umarani R) formulated the research work, helped in article writing and uploading; Third authors (Vanitha C, Anand T, Preetha G and Vijayabhama R) contributed in research work formulation and article editing. All authors have read and approved the research article.

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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TABLES

Table 1. Effect of polyphenols rich formulation on seedling physiological parameters @ 14 DAS

Treatments	Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedlings^{-1})	Vigour Index I	Vigour Index II
T_0 – Control (Un coated)	5.4^{b}	93^{a}	20.4^{d}	39.9^{c}	2.69^{e}	5608^{d}	250^{e}
T_1- Stock solution (No active ingredient)	5.6^{ab}	94^{a}	21.9^{cd}	42.2^{b}	2.78^{d}	6025^{cd}	261^{d}
T_2 – 100g/kg (5g ai)	5.7^{ab}	95^{a}	22.9^{bc}	43.2^{ab}	2.97^{c}	6280^{bc}	282^{b}
T_3 - 100g/kg (5.5g ai)	5.7^{ab}	95^{a}	23.1^{ab}	43.5^{ab}	2.99^{b}	6327^{ab}	284^{b}
T_4 -100g/kg (6g ai)	5.8^{a}	97^{a}	24.5^{a}	44.9^{a}	3.36^{a}	6732^{a}	326^{a}
T_5 -100g/kg (6.5g ai)	5.4^{b}	93^{a}	23.7^{ab}	43.1^{ab}	2.91^{bc}	6212^{b}	271^{c}
SEd	0.10	1.66	0.40	0.88	0.053	150.86	5.16
CD (P=0.05)	0.23	NS	0.89	1.97	0.119	336.14	11.49

Values followed by the same superscript letter(s) within a column are not significantly different at $P \leq 0.05$ according to LSD

FIGURES

Figure 1. GCMS analysis to identify the polyphenols in a polyphenol rich source

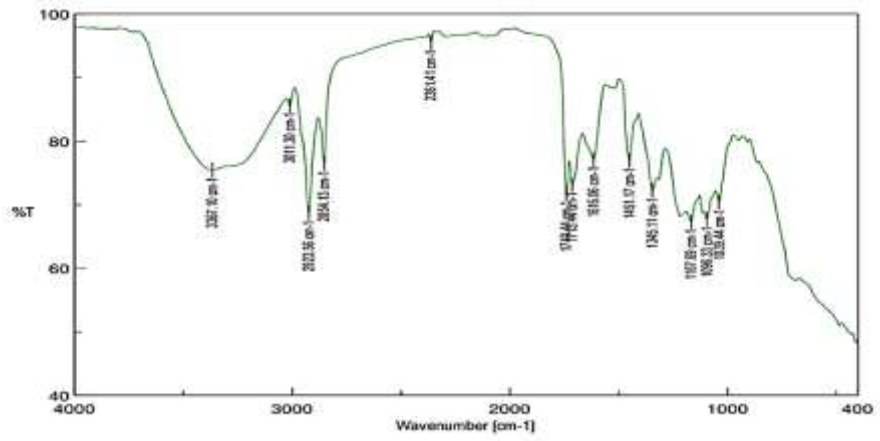
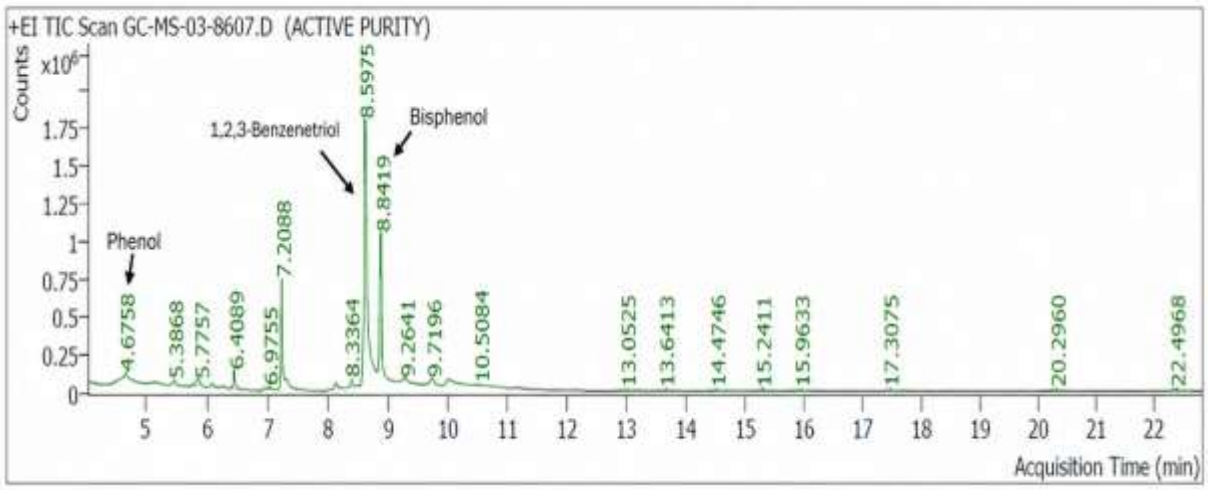
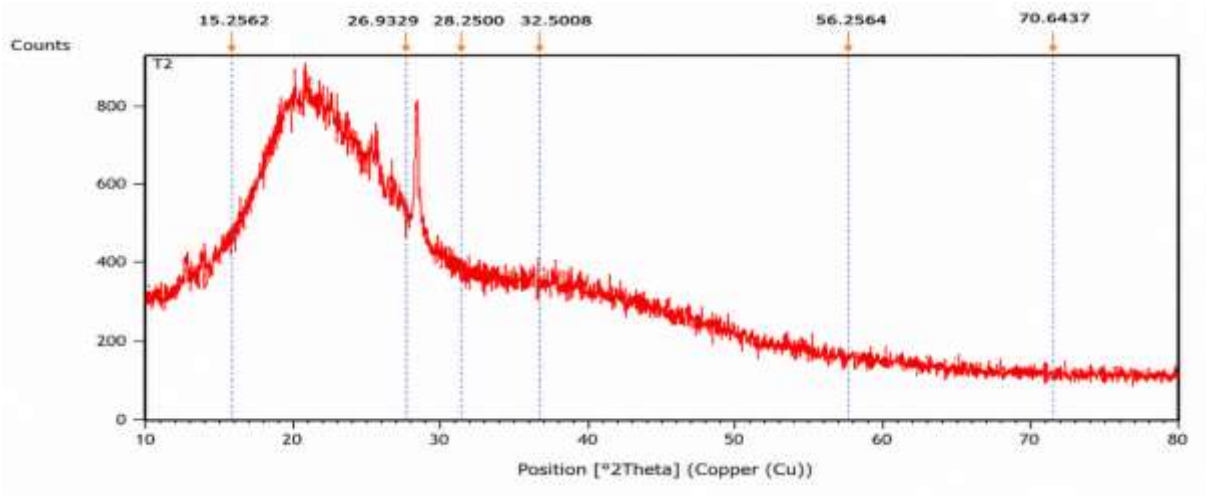


Figure 2. Characterization of polyphenols rich formulation



(a)



XRD pattern of polyphenols rich formulation **(b)**

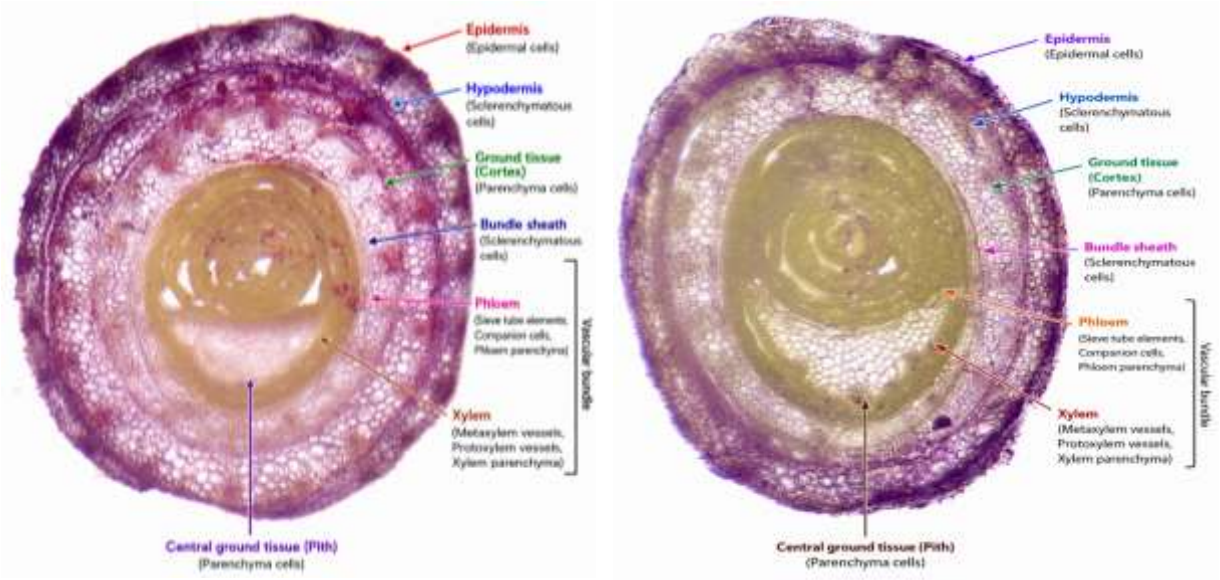
Figure 3. Effect of polyphenols rich formulation on seedling vigour of maize @ 14 DAS

T_0-Control

T_1-Stock solution (No ai) (100g/kg)

T_4-100g/kg (6g ai)

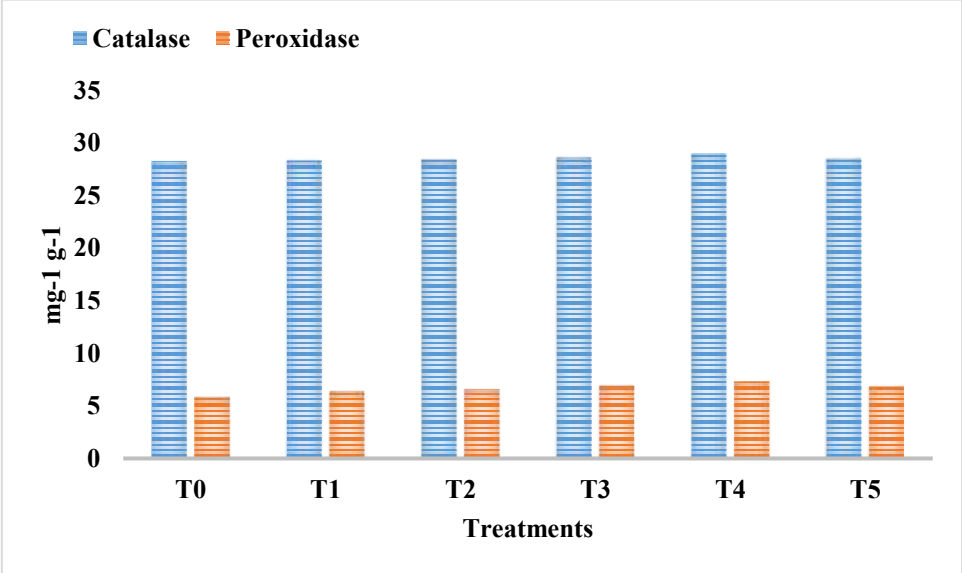
Figure 4. Cross section view of control and coated seedling @ 14 DAS



Control Seedling

Coated seedling-100g/kg (6g ai)

Figure 5. Effect of antioxidant enzyme activity of polyphenols rich formulation coated seeds



T_0 – Control (Uncoated), T_1 - Stock solution (No Active Ingredient) (100 g/kg), T_2 – 100 g/kg (5g ai), T_3 – 100 g/kg (5.5g ai), T_4 – 100 g/kg (6g ai), T_5 – 100 g/kg (6.5g ai)