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Comparative analysis of *Moringa oleifera* leaf extracts obtained using different extraction techniques and their influence on Cowpea seed germination

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A R T I C L E I N F O

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A B S T R A C T

Moringa oleifera leaf extract (MLE) has garnered significant attention as a natural plant growth stimulant, offering a cost-effective alternative to synthetic fertilisers due to its rich content of cytokinins, antioxidants, and essential macro- and micronutrients. Pressurised Hot Water Extraction (PHWE) has emerged as an environmentally friendly, rapid, and efficient technique for extracting these bioactive compounds from plant biomass. In this study, seven different MLEs were evaluated for their effects on cowpea (*Vigna unguiculata*) seed germination, using tap water as the control. These included MLEs obtained at 25, 50, 100, and 150 °C using PHWE, as well as extracts from a stirred batch system, a blended method, and a commercial biostimulant. Seeds treated with the control, 25 °C, and 150 °C extracts achieved 100 % germination, while the commercial biostimulant resulted in only 30 %. The 25 °C extract produced the longest hypocotyls (13.2 cm), whereas the commercial biostimulant yielded the shortest (2.1 cm). Secondary root emergence was highest (100 %) in seeds treated with the control, 25 °C, and stirred batch extracts. The 50 °C extract showed the greatest emergence of rudimentary leaves (43 %). Overall, the commercial biostimulant underperformed across all measured parameters. This study highlights the distinct influence of different extraction methods on the bioactivity of MLEs, underscoring their potential as sustainable growth-promoting agents in agriculture.

1. Introduction

Seed germination marks the beginning of a plant's life cycle. It is a complex physiological process that begins when an inactive, dry seed absorbs water, resulting in metabolic activation and the protrusion of the radicle from the seed coat (Makhaye et al., 2021). Germination comprises three prominent stages: rapid water uptake by the dry seed, metabolic reactivation, which involves protein synthesis and mobilisation of stored nutrients, and finally, the emergence of the radicle (Jhanji et al., 2024; Makhaye et al., 2021). This process is regulated by plant hormones such as abscisic acid (ABA) and gibberellic acid (GA), which play a significant role in seed germination and dormancy (Wang et al., 2024).

Understanding the germination process is crucial for crops such as cowpea (*Vigna unguiculata*), which plays a vital role in food and nutrition security in various parts of the world. Cowpea is a protein-rich legume primarily cultivated for its seeds, pods, and leaves, which humans consume. This versatile crop is also utilised as animal forage and can be incorporated into crop rotation to enhance soil fertility (Abebe and Alemayehu, 2022; Awika and Duodu, 2017). Global cowpea production has increased significantly over the past two decades, reaching 9.77 million tons in 2022. Africa accounts for the majority of cowpea production, contributing 96 % (Shevkani et al., 2025). Cowpea is also known for its health benefits, which can be attributed to its high content of polyphenols, antioxidants, fibre, minerals, vitamin B, and amino acids (Jayathilake et al., 2018).

Given its agricultural and nutritional importance, enhancing cowpea seed germination and early seedling development is essential for improving overall crop productivity. One promising approach involves the use of Moringa leaf extract (MLE), a plant biostimulant that has been shown to enhance seed germination and support early seedling growth (Khan et al., 2017; Sarmin, 2014; Soliman et al., 2017). Moringa leaf extract is regarded as an effective biostimulant due to its rich content in phytohormones, antioxidants, and essential nutrients that promote cell

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division, elongation, and stress tolerance, resulting in enhanced plant growth (Ahmad et al., 2019; Merwad, 2018; Zulfiqar et al., 2020).

Effectively obtaining these bioactive compounds largely depends on the extraction method employed. Conventional extraction methods for medicinal plants, such as Soxhlet extraction, maceration and liquid-liquid extraction, can be simple; however, they may exhibit lower extraction efficiencies, require large amounts of solvent, and often result in toxic solvent residues (Ameer et al., 2017; Chemat et al., 2019; Daso and Okonkwo, 2015). These limitations have increased the demand for modern, eco-friendly extraction technologies such as supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction and Pressurised Hot Water Extraction (PHWE) (Ameer et al., 2017; Rocchetti et al., 2019). Pressurised Hot Water Extraction utilises high pressure and temperature to enhance the solvent properties of water, enabling the efficient extraction of polar and semipolar compounds from plant biomass (Matshedisoet al., 2015; Nuapia et al., 2020a, 2020b).

Despite the growing interest in MLE as a biostimulant, limited research exists on how the extraction method, particularly PHWE at various temperatures, affects the biostimulant activity of the extract. This aspect is crucial because extraction parameters influence the recovery of bioactive compounds. This study aims to fill this gap by evaluating the effects of MLEs obtained through PHWE at 25, 50, 100, and 150 °C, as well as those prepared by blending, a stirred batch system, and a commercial biostimulant, on cowpea seed germination. The aim is to identify the optimal extraction conditions for enhancing seed germination and promoting early seedling growth.

2. Materials and methods

2.1. Chemicals

Diatomaceous earth, Folin–Ciocalteu reagent, gallic acid, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and methanol (HPLC grade) were purchased from Merck (Johannesburg, South Africa). Deionised water was produced through a Milli-Q Millipore Direct Q3 UV waters system (Massachusetts, USA).

2.2. Preparation of moringa leaf extract (MLE)

Mature fresh *Moringa oleifera* leaves were harvested, shade-dried at room temperature and pulverised at the Agricultural Research Council - Vegetables, Industrial and Medicinal Plants, Roodeplaat, Pretoria, South Africa.

2.2.1. Pressurised hot water extraction

Moringa leaf extracts were obtained using PHWE. The PHWE system consisted of a High-Performance Liquid Chromatography (HPLC) pump and a Gas Chromatography (GC) oven, which controlled the temperature and housed a stainless steel extraction cell. During extraction, deionised water was preheated to 80 °C and circulated through the system at a constant flow rate of 1.0 mL/min. A 2.5 g of *Moringa oleifera* leaf powder and 2.5 g of diatomaceous earth was packed into the extraction cell. The vessel was preheated to the desired extraction temperature (25, 50, 100, or 150 °C) for 15 min prior to extraction. The resulting extract was collected in 50 mL centrifuge tubes and stored at −18 °C.

2.2.2. Blended extraction

A blended extract was prepared by mixing 2.5 g of *Moringa oleifera* leaf powder with 50 mL of deionised water. The mixture was blended using a Bosch 600 W stick blender for 15 min and subsequently filtered through Whatman 1 filter paper.

2.2.3. Stirred batch extraction

For the stirred batch method, 2.5 g of *Moringa oleifera* leaf powder was stirred in 50 mL deionised water at 85–90 °C for 30 min in a closed system. The extract was cooled and filtered through Whatman 1 filter paper.

2.2.4. Preparation of commercial extract

A solution of a commercially available Moringa leaf biostimulant was prepared by mixing one part biostimulant with twenty parts deionised water.

2.3. Seed germination experimental design and treatment

Petri dishes (150 × 15 mm) were disinfected with ethanol and lined with Whatman 1 filter paper. Ten grade 1 cowpea seeds (Woolworths, Gauteng, South Africa) were placed into each dish and saturated with 9 mL of the respective treatments (control (C); PHWE at 25, 50, 100, or 150 °C; blended extract (WE); stirred batch extract (BO), and commercial biostimulant (CB)). Each dish was sealed in a zip-lock bag and incubated in a dark space at ambient temperature for 8 days. Fresh MLE was applied every 3 days to maintain moisture. Recorded parameters included germination percentage, radicle and hypocotyl lengths, emergence of rudimentary leaves, and development of the secondary root system.

2.4. Phytochemical studies

2.4.1. Determination of total phenolic content (TPC)

A Folin-Ciocalteu assay was used to determine the total phenolic content of the MLEs, using gallic acid as the reference standard (Pakade et al., 2013; Ralepele et al., 2021). To initiate the reaction, 200 µL of MLE was introduced into a freshly prepared mixture containing 750 µL of Folin-Ciocalteu reagent and 2 mL of 7.5 % sodium carbonate. The reaction mixtures were kept in the dark at room temperature for 2 h. Absorbance readings were measured at 765 nm using a UV–Vis spectrophotometer. Results were expressed as gallic acid equivalents (GAE) in g/100 g of extract (mean ± SD, $n = 3$).

2.4.2. Determination of total flavonoid content (TFC)

The total flavonoid content of the extract was determined using an aluminium chloride colourimetric assay (Pakade et al., 2013; Ralepele et al., 2021). Moringa leaf extract (0.3 mL) was added to a volumetric flask (10 mL). Subsequently, 0.3 mL of sodium nitrite (5 g/100 mL) was introduced, and the mixture was thoroughly mixed. After 5 min, 3 mL of aluminium chloride solution (1:100, wt/vol) was added, followed by an additional 6 min of incubation. Thereafter, 1 M sodium hydroxide solution (2 mL) was added, and the final volume was adjusted to 10 mL with deionised water. Absorbance was recorded at 510 nm using a UV–visible spectrophotometer, and flavonoid content was expressed as quercetin equivalents (QE) in g/100 g extract (mean ± SD, $n = 3$).

2.4.3. DPPH radical scavenging activity assay

The antioxidant activity was assessed using the DPPH radical scavenging assay (Pakade et al., 2013). Moringa leaf extract (0.1 mL) was mixed with 3.9 mL of 0.025 g/L DPPH in methanol. Samples were incubated in the dark at room temperature, and absorbance readings were recorded at 515 nm at 5-minute intervals for 120 min. The radical scavenging activity (RSA) percentage was calculated using Eq. (1).

$$\%RSA = \left(\frac{Abs_{Sample} - Abs_{Blank}}{Abs_{Control}}\right) X\ 100 \tag{1}$$

Where AbsControl was the absorbance of the DPPH methanolic solution, AbsBlank was the absorbance of the solvent, and AbsSample was the absorbance of the DPPH solution after adding the plant extract.

2.4.4. Data analysis

All results were reported as mean ± SD. Microsoft Excel for Mac

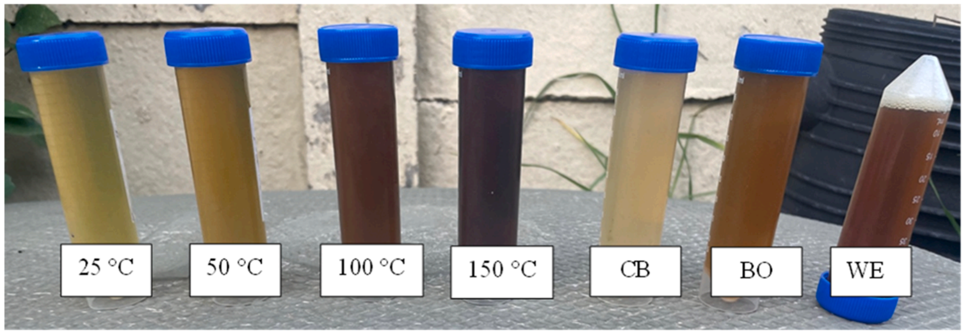

Fig. 1. Moringa Leaf Extracts obtained by various extraction techniques using water as a solvent.

Version 16.66.1 was used for graphical presentation and performing an Analysis of Variance (ANOVA). One-way and Two-way ANOVA interactions between the treatments were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Total phenolic content

Phenolic compounds, such as flavonoids and other secondary metabolites, are essential bioactive constituents in Moringa leaf extracts (Ralepele et al., 2021). These compounds are renowned for their antioxidant properties, which can influence various physiological processes in plants, particularly during germination and early growth stages (Makhaye et al., 2021). The MLE's total phenolic content (TPC) was assessed to determine its potential role in enhancing the germination and vigour of cowpea seeds.

The PHWE extracts were evaluated at 25, 50, 100, and 150 °C, with the TPC of the extracts being analysed. These extracts were compared to those produced using a stirred batch system (BO), blended (WE), and a commercial biostimulant (CB) (Fig. 1). The BO extract's TPC analysis revealed the highest TPC concentration with a concentration of 1304 ± 18.1 mgGAE/100 g, as shown in Fig. 2. These findings are consistent with previous studies by Llorent-Martínez et al. (2023) and Pop et al. (2022), which have shown that Moringa has a rich phenolic profile.

The results of the extracts obtained using PHWE indicate a relative increase in TPC with rising extraction temperatures, with the PHWE extracts reaching 953 ± 11.1 mgGAE/100 g at 150 °C, consistent with results reported by Matshedisoet al. (2015). Notably, the blended extract (WE) demonstrated a TPC of 951 ± 12.1 mgGAE/100 g, which is comparable to the TPC of PHWE extracts obtained at 100 °C (848 ± 12.0 mgGAE/100 g) and 150 °C (953 ± 11.1 mgGAE/100 g) (Fig. 2). This suggests that blending techniques, alongside high-temperature extraction techniques, can enhance the bioavailability of phenolic compounds, achieving results comparable to those obtained from higher-temperature processes. Among all the extracts, CB yielded the lowest TPC of 59 ± 1.2 mgGAE/100 g. This may be due to the extraction technique and the characteristics of the biomass used, such as the climate in which it was grown, cultivation practices, drying processes, and stability of the formulation (Ralepele et al., 2021). A one-way ANOVA to compare the TPC across different MLEs yielded a highly significant difference between the groups (F-value = 922.28, p-value < 0.05). The very low p-value of $2{,}3361\times10^{-17}$ indicates statistically significant differences in TPC among the extraction methods tested.

Variation in TPC is relevant because phenolic compounds are known contributors to antioxidant activity, which plays a key role in seed germination and early seedling development (Lawag et al., 2023; Vuolo et al., 2019). Extracts with higher TPC often display greater antioxidant capacity, which may help reduce oxidative stress during germination and therefore supporting growth-promoting effects (Ahammed et al.,

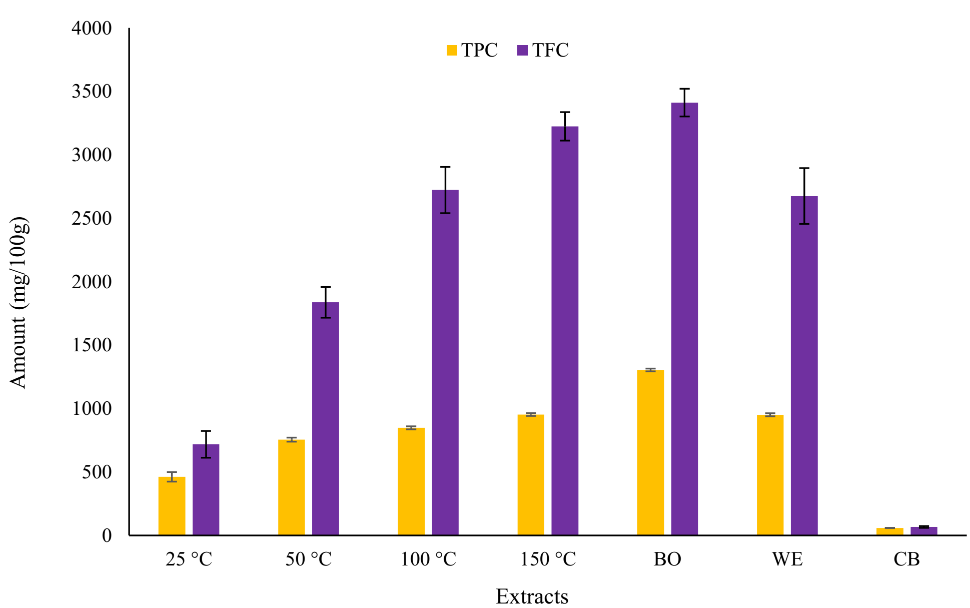

Fig. 2. Total Phenolic Content and Total Flavonoid Content of Moringa leaf extracts (n = 3). BO (Stirred batch system), WE (Blended extract), CB (Commercial Biostimulant).

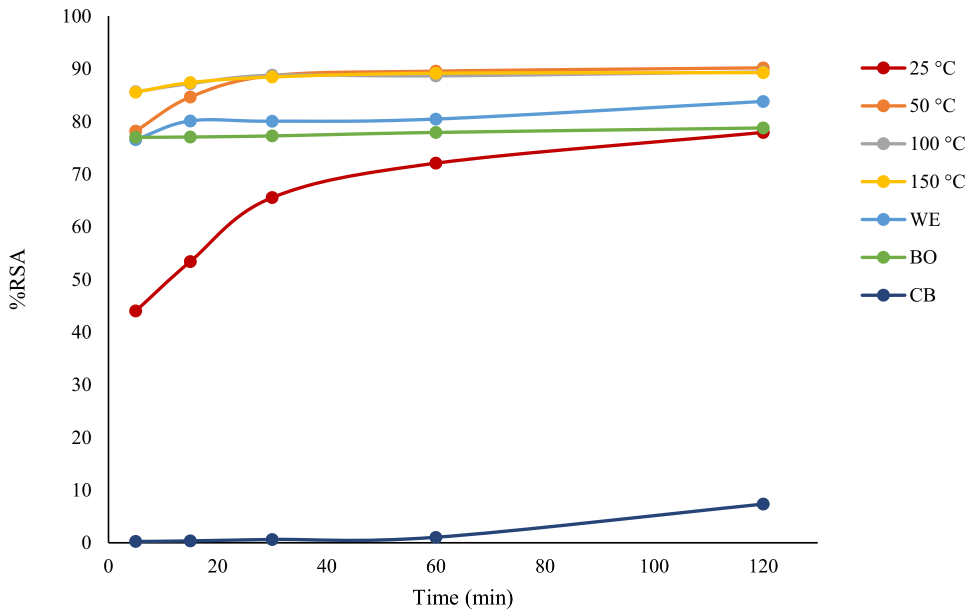

Fig. 3. Time study of Radical Scavenging Activity percentage (%RSA) of Moringa leaf extracts incubated at 5, 15, 30, 60 min and 120 min ($n = 3$). BO (Stirred batch system), WE (Blended extract), CB (Commercial Biostimulant).

2018; Kumar et al., 2023; Mencin et al., 2021).

3.2. Total flavonoid content

As shown in Fig. 2, the trend observed closely mirrors that of the TPC results. The BO extract showed the highest flavonoid content (3411 ± 109.22 mgQE/100 g), followed by the 150 °C PHWE extract (3224 ± 112.2 mgQE/100 g). A one-way ANOVA revealed a highly significant effect of extraction method on flavonoid content as indicated by a high F-value of 162, and a p-value below 0.05. Extracts with high phenolic and flavonoid content possess potent antioxidant properties. Biostimulants, such as MLE, are rich in phenolic and flavonoid compounds and, thus, antioxidants (Vergara-Jimenez et al., 2017). Phenolic and flavonoid compounds in MLE play a crucial protective role during seed germination by mitigating oxidative stress (Ahammed et al., 2018). Germination is associated with increased metabolic activity, resulting in the production of reactive oxygen species (ROS). These compounds help neutralise ROS, therefore preserving cellular integrity and supporting successful germination (Li et al., 2022). For example, phenolic and flavonoid compounds can enhance the synthesis of nucleic acids and proteins that are vital for cellular function and seedling development (Wang et al., 2020). Kumar et al. (2018) further demonstrated that flavonoids improve seed protection and germination.

3.3. Effect of incubation time on antioxidant activity

The radical scavenging activity of the seven MLEs was evaluated using a DPPH assay, as illustrated in Fig. 3. The percentage radical scavenging activity (%RSA) for each extract was monitored over a two-

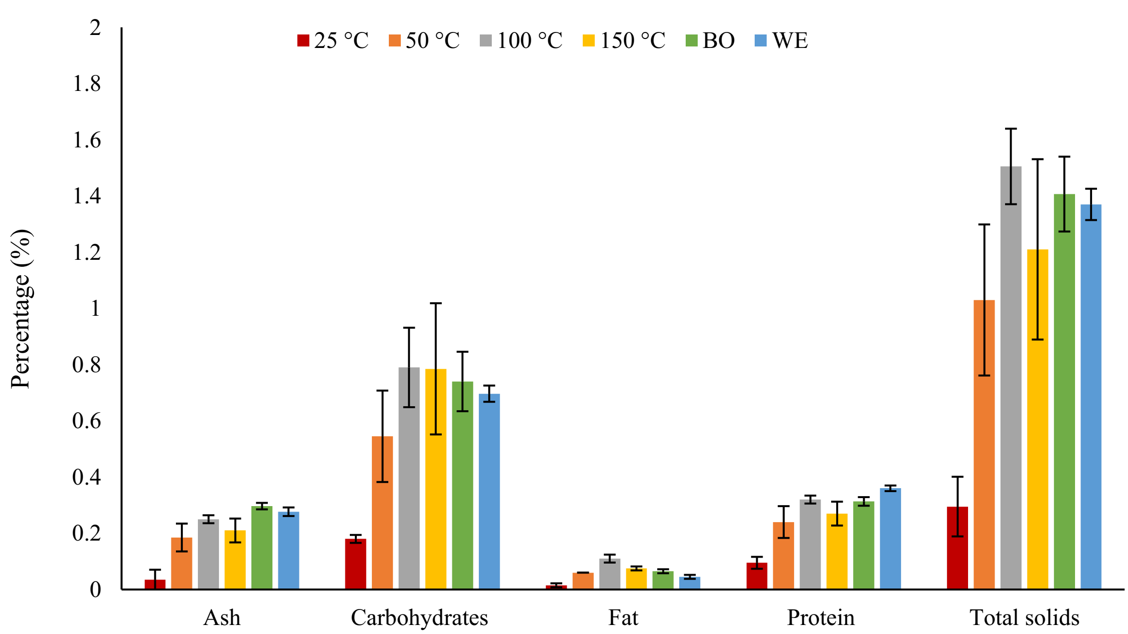

Fig. 4. Physiochemical composition of Moringa leaf extracts, ($n = 3$), BO (Stirred batch system), WE (Blended extract), CB (Commercial Biostimulant).

hour period. This assay measures the ability of antioxidants within the extracts to neutralise DPPH free radicals by donating hydrogen atoms, therefore converting DPPH into its non-radical form, DPPH—H. Consequently, a higher %RSA reflects a greater antioxidant capacity in the MLEs (Chibuye et al., 2024; Ralepele et al., 2021).

A two-way ANOVA with replication revealed a statistically significant difference in antioxidant concentrations across all seven extracts ($p < 0.05$, F-value = 250). All extracts, except the CB extract, showed relatively high antioxidant activity, with %RSA values exceeding 70 % after two hours of incubation. In contrast, the CB extract demonstrated a much lower %RSA of only 7 %. The PHWE extracts obtained at 50, 100, and 150 °C exhibited the highest antioxidant capacity, each achieving % RSA values of 90 %, 89 %, and 89 %, respectively. This strong radical scavenging performance is likely due to the abundance of polyphenolic compounds extracted through PHWE (Matshedisoet al., 2015). Conversely, the 25 °C extract showed a more gradual increase in antioxidant activity, starting from 44 % at 5 min to 78 % after 2 h. These findings are consistent with previous reports by Matshedisoet al. (2015), which also showed lower antioxidant activity at 25 °C and higher values at elevated temperatures.

The results, as illustrated in Fig. 3, highlight the importance of incubation time. This study found that incubation time has a significant effect on the total antioxidant concentration in the extracts ($p < 0.05$). An increase in %RSA was observed between 5 and 30 min across all extracts, after which the values plateaued, indicating no significant changes beyond this point. This trend supports the common practice of allowing at least 30 min of incubation in DPPH assay studies (Bhalla et al., 2021; Natsir et al., 2019). These findings demonstrate that both incubation time and extraction methods significantly influence the antioxidant capacity. Notably, at higher temperatures, particularly 150 °C, produced extracts with superior antioxidant capacity, achieving peak performance within the first 30 min.

3.4. Physiochemical composition of Moringa leaf extracts

A standard approach to evaluating the nutritional content of food and plant-based products involves analysing ash, carbohydrates, fat, protein, and total solids. Moringa leaves are renowned for their high nutrient content, and Fig. 4 illustrates the physicochemical composition of the seven MLEs examined in this study. Ash content, which reflects the total mineral content in the MLE, provides insight into the potential availability of essential minerals that support enzymatic activity and cellular processes (Iskandar et al., 2020; Yuan et al., 2022). Among the extracts, the BO extract exhibited the highest ash content (0.30 %), followed by the blended extract (WE) at 0.28 % and the 100 °C extract at 0.25 %.

The 100 °C extract proved to be a good source of carbohydrates, fats, proteins, and total solids, showing the highest concentration of these nutrients. Carbohydrates provide the immediate energy required for germination and the early stages of plant growth (Chibbar et al., 2016). Proteins in the extract provide amino acids necessary for building new proteins, thereby aiding in the development of the seedlings' structure and metabolism (Vujetić et al., 2024). A high total solid content signifies a nutrient-rich profile, suggesting that the extract may support various metabolic activities during germination. The physicochemical composition of the CB extract showed very low concentrations ranging from 0.01 % to 0.08 %; hence, it cannot be seen in Fig. 4. Among the seven extracts, the 25 °C and CB extracts had the lowest overall nutrient content.

Wardana et al. (2022) reported an ash content of 8.36 % for an MLE obtained *via* microwave extraction, while Biel et al. (2017) reported crude ash and protein contents of 17.54 % and 13.64 %, respectively, using water extraction at 95–100 °C. Both studies expressed their results on a dry matter basis. In contrast, this study reports values on a wet basis, which are naturally lower due to the moisture content retained in the samples.

Table 1
pH and electrical conductivity of Moringa leaf extracts.

Treatment	pH	EC (mS/cm)
25 °C	5.17 ± 0.12	1.046 ± 0.18
50 °C	5.20 ± 0.08	1.615 ± 0.55
100 °C	5.52 ± 0.01	3.194 ± 0.45
150 °C	5.38 ± 0.16	2.851 ± 0.90
BO	5.00 ± 0.87	4.283 ± 0.05
WE	5.78 ± 0.09	3.279 ± 0.04
CB	6.23 ± 0.21	0.285 ± 0.00

Mean ± SD, (n = 3), BO (Stirred batch system), WE (Blended extract), CB (Commercial Biostimulant).

3.5. Effect of pH and electrical conductivity on germination

To ensure that the applied extracts did not inhibit the germination rate, the pH and electrical conductivity (EC) of the extracts were measured (Table 1). This is important because the pH of the environment has a significant influence on seed germination and seedling development. In highly acidic conditions, essential nutrients such as calcium, phosphorus, and magnesium become less bioavailable (Johan et al., 2021). Additionally, metals such as manganese and aluminium become more soluble and potentially toxic to seed germination and plant growth (Long et al., 2024).

Cowpea seeds, in particular, prefer slightly acidic to neutral conditions for optimal germination and growth. The ideal pH range for growing cowpeas is approximately 5.5 to 6.5 (Adekiya et al., 2024; Osipitan et al., 2021). This pH range ensures nutrient availability, therefore promoting healthy seedling development and successful yields (Barnett, 2023; Coffigniez and Briffaz, 2023). As shown in Table 1, the pH values of the MLEs ranged from 5.00 to 6.23, which falls within the recommended range for cowpea cultivation (Adekiya et al., 2024). The BO extract was the most acidic (5.00 ± 0.87), while the CB extract was the least acidic (6.23 ± 0.21), indicating that all extracts are generally suitable for promoting germination without risking pH-related inhibition.

Based on TPC, TFC, and DPPH assay results, the BO extract exhibited the highest polyphenol content compared to the other extracts. This may be partially attributed to its lower pH (5.00 ± 0.87), as phenolic compounds are known to remain more stable in acidic environments due to their reduced susceptibility to oxidation and degradation (Ercoli et al., 2021; Pasquet et al., 2024; López-Parra et al., 2024). However, phenolic recovery is not only determined by pH. The PHWE extracts obtained at higher temperatures (100 °C and 150 °C) had slightly higher pH values (5.52 ± 0.01 and 5.38 ± 0.16, respectively), yet still showed high TPC, suggesting that elevated temperatures enhance polyphenol release from the plant matrix. In contrast, the more acidic PHWE extracts obtained at lower temperatures (25 °C and 50 °C) had lower TPC values, likely due to reduced extraction efficiency at those temperatures despite their favourable pH.

The electrical conductivity (EC) of an MLE provides valuable insights into its concentration of minerals and nutrients, as dissolved ions influence EC. Previous studies have reported that plant extracts rich in antioxidants often exhibit higher EC values (Parijatham Kanchana et al., 2023; Saad et al., 2015). In this study, the BO extract recorded the highest EC at 4.283 ± 0.05 mS/cm (Table 1), consistent with its high TPC and TFC. In contrast, the extract obtained at 25 °C, excluding the CB extract, showed the lowest EC (1.046 ± 0.18 mS/cm). These results highlight the impact of extraction temperature on the concentration of phytochemicals, minerals, and nutrients (Matshedisoet al., 2015).

Interestingly, despite being processed at room temperature, the blended extract exhibited a significantly high EC of 3.279 ± 0.04 mS/cm, comparable to the extract obtained at 100 °C (3.194 ± 0.45 mS/cm). This suggests that blending Moringa leaf powder potentially enhances the concentration of phytochemicals and minerals by increasing the surface area, thereby exposing more leaf material to water.

Table 2
Germination percentage of cowpea seeds treated with Moringa leaf extracts over 8 days.

Treatment	Germination percentage (%)					
	Day 2	Day 3	Day 4	Day 6	Day 8	Germination rate*
Control	60	80	90	100	100	77.50
25 °C	60	70	70	80	100	66.25
50 °C	50	50	50	50	70	46.25
100 °C	40	60	70	80	90	54.50
150 °C	40	70	80	100	100	71.25
BO (Stirred Batch)	30	60	90	90	90	67.50
WE (Blended)	20	50	50	60	70	45.00
CB (Commercial Biostimulant)	0	0	0	20	30	8.75

* Germination rate calculated over the 8 days.

Additionally, the smaller particle size resulting from blending may improve the bioavailability of nutrients, facilitating better absorption by the seeds. Moreover, blending promotes a more uniform distribution of phytochemicals throughout the powder, reducing the likelihood of clumping or uneven dispersion in water.

3.6. Germination studies

3.6.1. Germination percentage of cowpea seeds treated with MLE

Seed germination is a vital stage in the plant life cycle and has direct implications for agricultural productivity and food security (Farooq et al., 2021). To assess the biostimulant potential of the MLEs, the germination performance of cowpea seeds treated with each of the seven extracts was evaluated.

A two-way ANOVA indicated that both time and extract type had a statistically significant effect on the germination rate ($p < 0.05$).

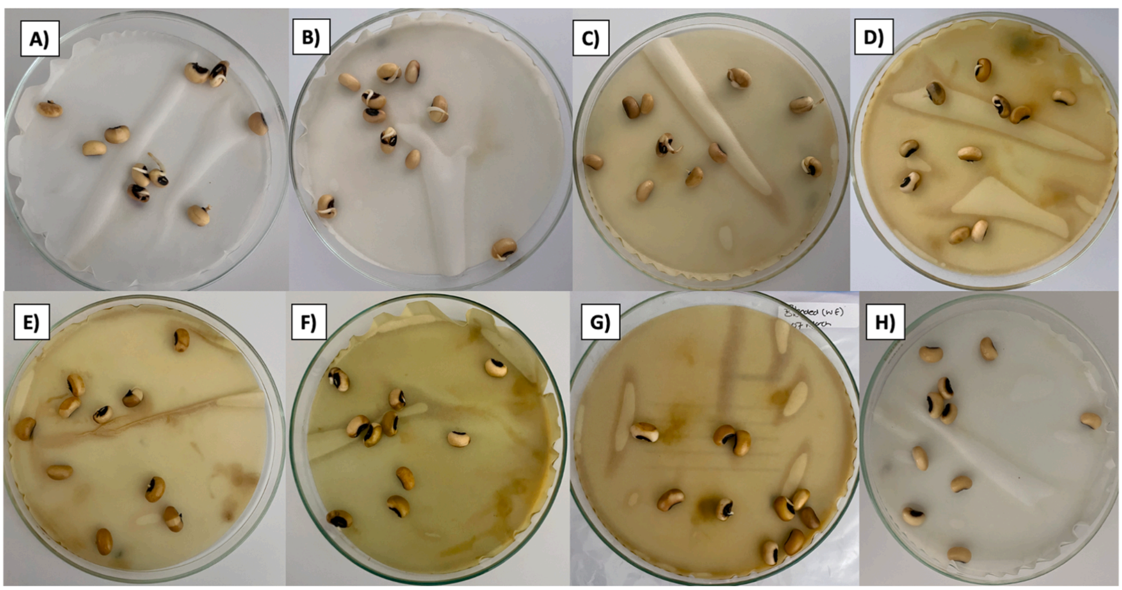

Fig. 5. Radical protrusion after 2 days of being soaked in respective treatment. Seeds soaked in A) tap water (control), PHWE at B) 25 °C, C) 50 °C, D) 100 °C, E) 150 °C, the F) stirred batch system extract (BO), G) blended extract (WE) and the H) commercial biostimulant (CB).

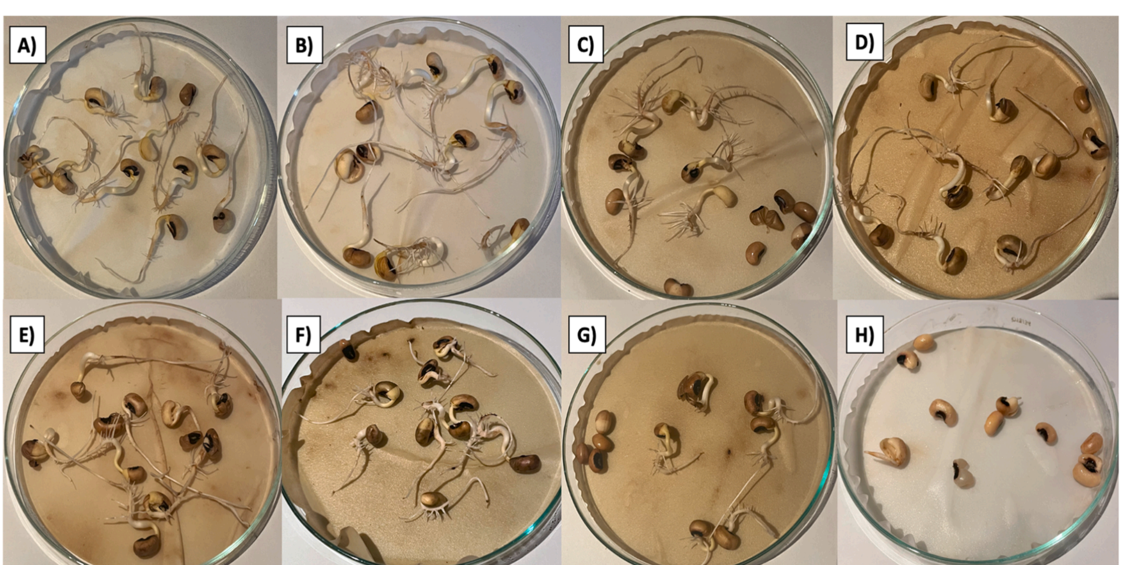

Fig. 6. Cowpea germination test after 6 days. Seeds soaked in A) tap water (control), PHWE at B) 25 °C, C) 50 °C, D) 100 °C, E) 150 °C, the F) stirred batch system extract (BO), G) blended extract (WE) and the H) commercial biostimulant (CB).

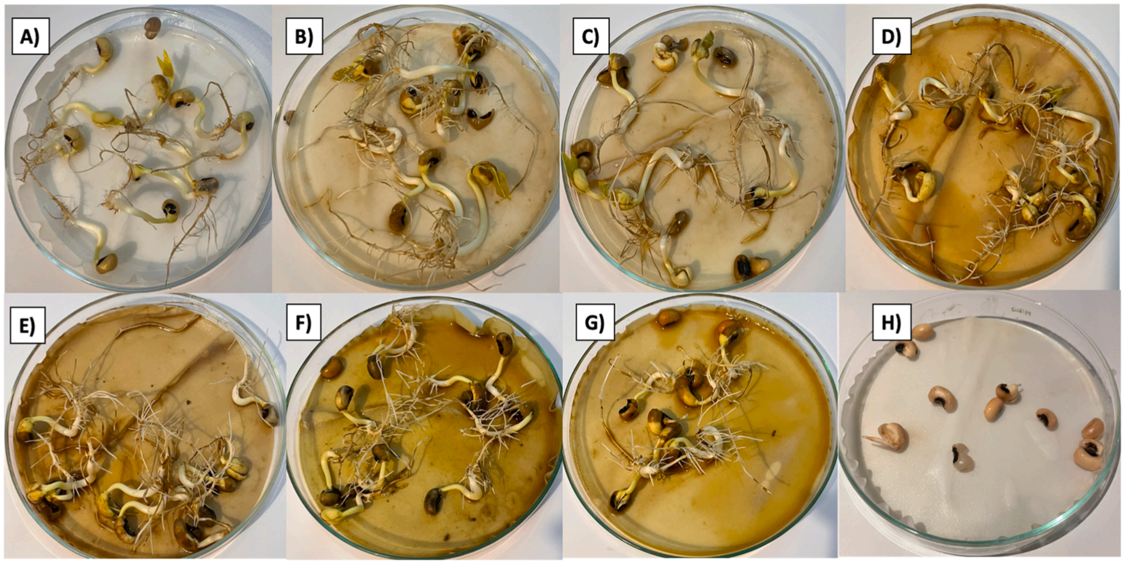

Fig. 7. Cowpea germination test after 8 days- formation of rudimentary leaves. Seeds soaked in A) tap water (control), PHWE at B) 25 °C, C) 50 °C, D) 100 °C, E) 150 °C, the F) stirred batch system extract (BO), G) blended extract (WE) and the H) commercial biostimulant (CB).

Germination percentages over the 8-day period are presented in Table 2. As shown in Table 2 and Fig. 5, by day 2, 60 % of seeds had germinated in both the control and 25 °C extract treatments. In contrast, the lowest germination rates were observed for the blended extract (20 %) and CB extract, which showed no germination.

At the end of the germination experiment, the control group (treated with tap water) exhibited the highest average germination rate at 77.50 %, outperforming the 150 °C extract, which achieved a germination rate of 71.25 %. The superior performance of the control group suggests that while MLEs contain bioactive compounds, they may also introduce certain phytochemicals or mineral salts that, when present in high concentrations or highly bioavailable forms, could inhibit germination (Phiri and Mbewe, 2010; Soliman et al., 2017; Zakaria and Razak, 1990). This is consistent with observations by Soliman et al. (2017), those who found that control seeds outperformed those treated with higher concentrations of MLE in *Vicia faba*.

Interestingly, although the 150 °C extract did not outperform the control, it did achieve a comparable final germination percentage (100 %) and a relatively high germination rate. This suggests that high temperature PHWE may have reduced the concentration of certain inhibitory compounds through thermal degradation, while simultaneously enhancing the extraction of heat-stable, germination-promoting compounds. Furthermore, elevated temperatures may disrupt plant cell wall structures, improving the release and bioavailability of beneficial nutrients (de Lima Marsiglia et al., 2023; Khoza et al., 2014; Matshedisoet al., 2015). Therefore, the extract obtained at 150 °C appears to have achieved a functional balance, by minimising inhibitory effects whilst maximising the availability of germination-promoting compounds. This could explain its superior performance compared to other MLE treatments.

By day 6 of the experiment, secondary root formation was observed (Fig. 6), with both the control and the 150 °C treatments achieving 100 % germination. No further germination was noted in the BO extract group, which remained at 90 %. By the end of the experiment (day 8), only the control, 25 °C and 150 °C treatments achieved 100 % germination. The 100 °C and BO treatments followed closely at 90 % while the

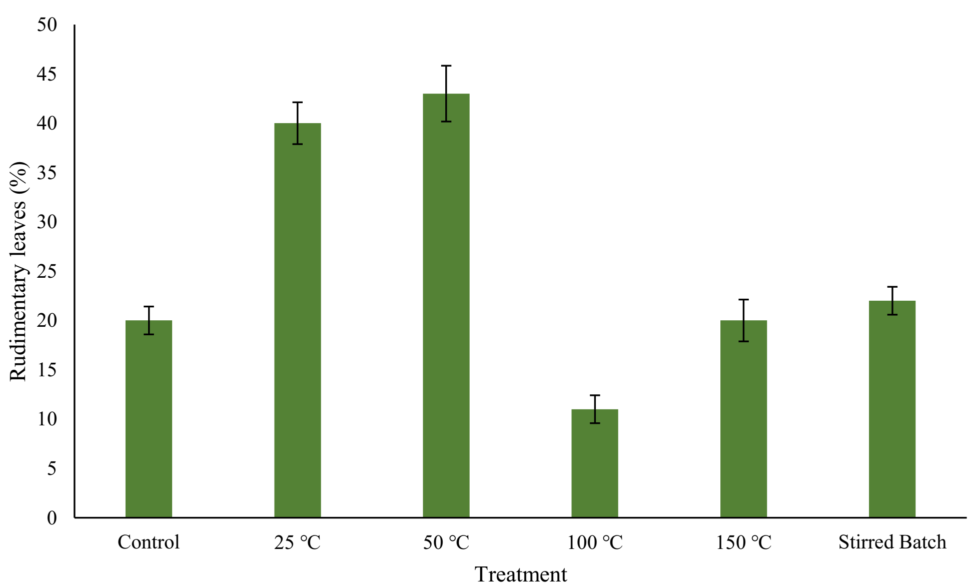

Fig. 8. Emergence of rudimentary leaves on day 8. Seeds soaked in tap water (control), PHWE at 25 °C, 50 °C, 100 °C, 150 °C, and the stirred batch system extract.

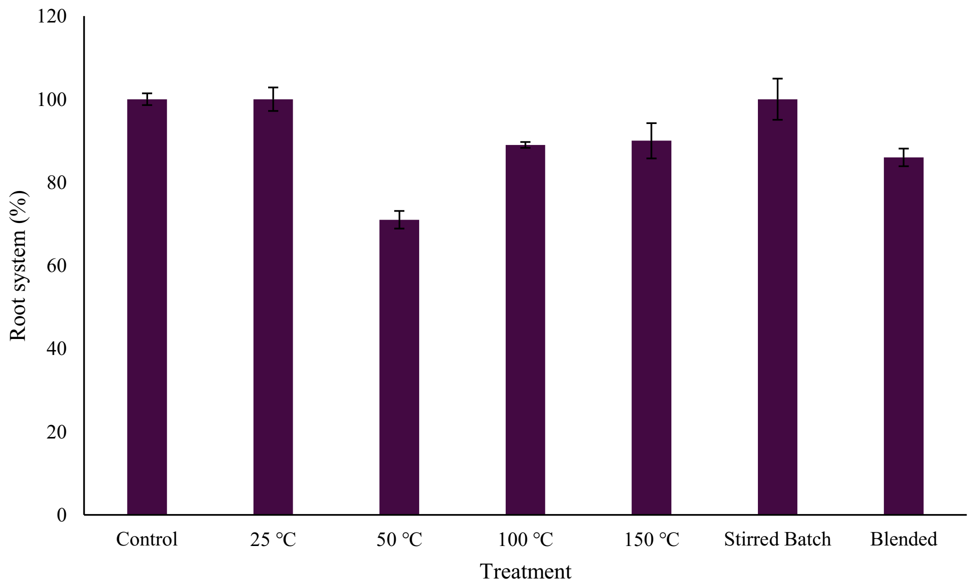

Fig. 9. Emergence of the root system on day 8. Seeds soaked in tap water (control), PHWE at 25 °C, 50 °C, 100 °C, 150 °C, the stirred batch system extract, blended extract and the commercial biostimulant.

50 °C and blended (WE) extracts reached 70 %. Once again, the CB extract recorded the lowest germination percentage of 30 %.

3.6.2. Emergence of rudimentary leaves

The emergence of rudimentary leaves was observed on day 8 of the germination experiment, and results varied across treatments (Fig. 7 and Fig. 8). The MLE extracted at 50 °C resulted in the highest percentage of rudimentary leaf formation (43 %), followed by the 25 °C extract, with 40 % of seeds showing rudimentary leaf emergence. In contrast, no rudimentary leaves were observed in seeds treated with the blended (WE) or CB extracts. This trend may indicate an increased concentration of shoot-promoting phytohormones, such as cytokinins, in the 25 °C and 50 °C MLEs (Ullah et al., 2024).

3.6.3. Formation of the root system

By the end of the experiment (day 8), 100 % of the seedlings in the control, 25 °C extract, and BO extract treatments exhibited secondary root development (Fig. 7& Fig. 9). This was followed by the 150 °C, 100 °C, and the blended (WE) extracts, where 90 %, 89 %, and 86 % of seedlings, respectively, developed a secondary root system. Several studies have reported that seed priming with MLE can enhance root vigour and length, particularly during the early developmental stages (Faried et al., 2024; Khan et al., 2017; Phiri and Mbewe, 2010; Yuniati et al., 2023). However, these effects appear to be concentration-dependent. In legumes such as cowpea, higher concentrations of MLE were associated with reduced root length (Phiri, 2010; Phiri and Mbewe, 2010). This suppression is thought to be linked to certain bioactive compounds in MLE, such as catechol and benzofuran, which may interfere with early root development (Sangeetha, 2023).

The MLE extracted at 50 °C resulted in the highest emergence of rudimentary leaves (43 %) (Fig. 8), but it showed reduced development of the secondary root system (Fig. 9). This suggests that there may be a

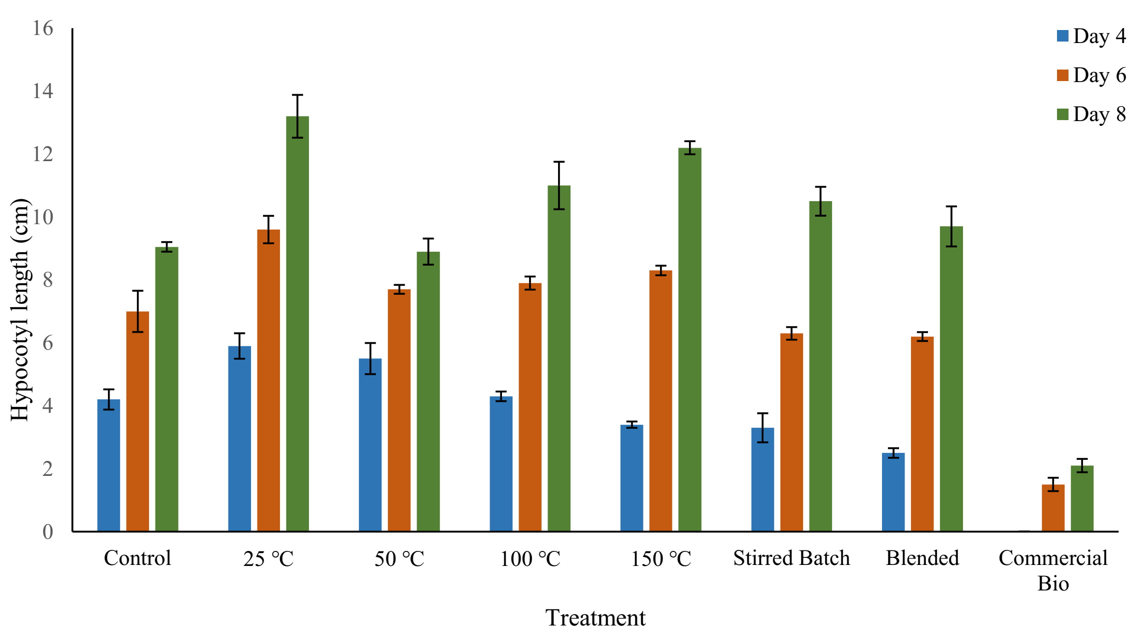

Fig. 10. Effect of Moringa leaf extracts on hypocotyl lengths (cm) of cowpea.

potential phytohormone imbalance in the 50 °C extract, whereby the extract may have retained or recovered higher levels of shoot-promoting compounds such as cytokinins. Cytokinins, such as zeatin, are known to stimulate leaf emergence and shoot growth while inhibiting root development, resulting in a lower root-to-shoot ratio (Ullah et al., 2024). Moderate extraction temperatures, such as 50 °C, may gently release heat-sensitive compounds from the plant biomass without causing degradation.

3.6.4. Hypocotyl and radicle length

The hypocotyl length (cm) increased across all treatments over the 8-day period. The 25 °C MLE treatment produced the longest average hypocotyl length (13.24 cm), followed by the 150 °C MLE (12.23 cm) and the 100 °C MLE (11.00 cm) (Fig. 10). Although the control group exhibited the highest germination rate on days 2 and 3 (Table 2), it did not result in the longest average hypocotyls (9.05 cm). Notably, the 150 °C MLE demonstrated the second-highest germination rate (71 %) and the second-longest hypocotyl length, indicating its superior performance compared to the other extracts.

Conversely, the CB extract was least effective, producing both the shortest hypocotyl length and the lowest germination rate. Similar observations have been reported by Phiri and Mbewe (2010), who found that while MLE increased the hypocotyl length by 16.6 % in groundnuts. Sarmin (2014) also noted that a 25 % MLE resulted in the longest hypocotyl length. Their study revealed a general trend in which an increase in MLE concentration resulted in a decrease in hypocotyl length, suggesting that higher concentrations may inhibit shoot elongation.

Overall, seeds treated with the CB extract exhibited delayed germination, possibly indicating phytotoxicity that can inhibit seed development (Makhaye et al., 2021). In contrast, the 25 °C MLE provided a more balanced physiological response. All seeds for this treatment had germinated by day 8, and the extract produced the longest average hypocotyl length. It also resulted in 100 % of seedlings developing a secondary root system and was the second highest performing treatment in terms of rudimentary leaf emergence. These results suggest that the 25 °C extract may offer an optimal combination of bioactive compounds that support both shoot and root development during early seedling growth.

4. Conclusion

The study demonstrates that cowpea germination is significantly influenced by Moringa leaf extract. Notably, the extract obtained at 25 °C consistently outperformed the other extracts, achieving 100 % germination, the longest hypocotyl length (13.2 cm), and the highest rate of secondary root emergence (100 %). In contrast, the CB extract underperformed in every parameter measured. These findings highlight the potential of Moringa leaf extract as a natural, effective biostimulant to enhance seed germination and early seedling growth. The superior performance of the 25 °C extract highlights the importance of optimising extraction conditions to maximise the benefits of natural plant extracts in agricultural applications.

CRediT authorship contribution statement

Boitumelo Setati: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Salmina Mokgehle:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Luke Chimuka:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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