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Fortification Of Phenolics, Antioxidant Activities and Biochemical Attributes of Radish Root Treated with *Moringa Oleifera* Extracts Based Senps

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ABSTRACT

The present study was planned to induce growth, phenolic content, antioxidant activities, and biochemical attributes of Radish (*Raphanus sativus* L.) root by seed priming with Plant Leaf Extracts (PLEs). The growth of Radish was monitored while growing seeds primed three *Moringa Oleifera* Extracts Based Selenium Nanoparticles (MEBSEN) including (MEBSEN-I) low extract to selenium ratio, (MEBSEN-II) with moderate extract to selenium ratio, and (MEBSEN-III) with high extract to selenium ratio along with Simple *Moringa* leaf extract (MoLE). The plants were harvested after emergence (DAE) and roots were subjected to proximate, biochemical and antioxidant analysis. It was observed that priming with PLEs not only induced the growth physiology but also improved total phenolic content (TPC), radical scavenging capacity (DPPH IC₅₀) and reducing power of radish root. Overall, seed priming with 3 % of MoLE for 6 hours was found to be the most effective to induce optimum growth with improved biochemical and antioxidant attributes. The growth behavior in primed radish plant seeds with (MEBSEN-I), (MEBSEN-II), and (MEBSEN-III) along with Simple *Moringa* leaf extract (MoLE) were regularly monitored whereas proximate composition, biochemical attributes and antioxidant potential was assessed after Antioxidant activities 60 days of emergence. Results explored that PLE based seed

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priming greatly affect growth physiology consequently in carotenoids proved total phenolic content (TPC), radical scavenging capacity (DPPH) and reducing power of radish root. Overall, seed bio-priming with 3% of MoL for 6h was found to be the most effective to optimize growth with improved biochemical and antioxidant attributes. In further, seed bio-priming with crude extracts of selected plant leaves may provide viable bioactive fortification choice.

Keywords— Fortification; Radish Root; Seed Priming; Plant leaf extracts; Antioxidant activities

1. INTRODUCTION

Global population growth continues to threaten food security, especially in developing nations such as Pakistan where agricultural demands are escalating rapidly (1,4). Novel farming techniques have been implemented to boost yields substantially (Ge et al. 2010; Tan et al. 2005). However, this progress has coincided with heavy reliance on synthetic fertilizers and chemicals, resulting in nutrient deficiencies alongside dangerous residue buildup in crops—particularly in root vegetables consumed entirely like radish (Coats 1994; Walorczyk et al. 2015) (2,3).

Vegetables naturally supply critical micronutrients, including bioactive compounds and antioxidants essential for preventing chronic diseases. Phenolics, the predominant phytochemical group, play pivotal roles in plant metabolism and human health (3,5) (Masondo et al. 2016). Regrettably, agrochemicals erode these beneficial constituents while introducing toxic contaminants that pose serious health risks when ingested (4,6) (Darko and Akoto 2008; Mushtaq et al. 2012).

Organic agriculture offers a promising solution, reducing chemical contamination, preserving ecosystems, and leveraging sustainable inputs (5,7) (Raghav 2006). Radish (*Raphanus sativus* L.) from the Brassicaceae family—locally called "muli"—features edible roots with documented therapeutic value, utilized in Chinese medicine for over 1400 years (6,8) (Duan et al. 2006). Its nutritional profile includes powerful antioxidants, phenolics, vitamins, and related compounds that deliver significant health advantages (7,9) (Goyeneche et al. 2015; Gutiérrez and Perez 2004).

While conventional practices degrade these qualities through residue accumulation, organic strategies hold potential for nutritional enhancement (8,10,11,12,13,14,15) (Lugasi et al. 2005). Despite widespread organic adoption, few studies explore selenium-based biofortification's impact on radish root quality (9,16,17,18). This research delivers detailed insights into using *Moringa oleifera* leaf extract-derived selenium for root treatment, specifically evaluating improvements in phenolic levels, antioxidant capacity (DPPH, FRAP assays), and key biochemical parameters (enzymes, nutrient uptake) of radish roots (10,19,20,22).

2. MATERIALS AND METHODS

2.1 Procurement of seeds and plant material

Radish seeds were obtained from Ayub Agricultural Research Institute (AARI),

Faisalabad. Young *Moringa oleifera* leaves were gathered from University of Agriculture Faisalabad Botanical Garden, rinsed thoroughly (2-3 times) with distilled water, and prepared for extract processing.

2.2 Development of Moringa-selenium formulation (MOSe)

Moringa leaves were ground with water (10 kg/L) using a mechanical extractor (Price, 2007). The homogenate was filtered through muslin cloth, then centrifuged ($8000 \times g$, 15 min). Clear supernatant (100% extract) had sodium selenite (1-5 mM) incorporated, incubated at 40°C for 2 hours with magnetic stirring to yield MOSe complexes. Working solutions (3% and 5%) were diluted from this stock.

2.3 Radish growth and treatment protocol

Uniform radish seeds were germinated in greenhouse pots at Agronomy Department, University of Agriculture, Faisalabad. Thirty days post-emergence, roots received MOSe treatments (3%, 5%) through soil drenching and foliar application for 6 or 9 hours under aerated conditions. Growth chamber maintained $25 \pm 2^\circ\text{C}$ and 70% relative humidity.

2.4 Harvest and sample preparation

Roots were collected at 60 days after emergence, cleaned with distilled water, surface-dried with blotting paper, and divided for antioxidant assays and biochemical profiling.

2.5 Phenolic compound extraction

Fresh roots (5 g) were pulverized in 80% methanol (1:10 w/v ratio), sonicated (30 min), centrifuged ($10,000 \times g$, 10 min), and supernatants preserved at 4°C.

2.6 Quantification of total phenolics (TPC)

Total phenolic content utilized Folin-Ciocalteu methodology calibrated against gallic acid standards. Values reported as mg GAE/g fresh root tissue.

2.7 FRAP antioxidant capacity

Reducing potential with root extracts at 5, 7.5, 10 mg/mL concentrations. Absorbance recorded at 593 nm relative to vitamin C standard.

2.8 DPPH free radical scavenging

Radical scavenging efficiency used DPPH

$$\% \text{ Inhibition} = [(ADPPH - A_{\text{Sample}}) / ADPPH] \times 100$$

IC₅₀ determined from dose-response curves plotting inhibition versus extract concentration.

2.9 Data processing

All treatments performed in three biological replicates. Results presented as mean $\pm$ standard deviation. Statistical significance ($p < 0.05$) determined by ANOVA with post-hoc Duncan's test via Statistix 9.0.

3 Results and Discussion

Root treatment with MOSe formulations produced marked improvements ($p < 0.05$) in extractable bioactive yields from radish roots (Figure 1). Differences in bioactive recovery (g/100g fresh weight) correspond to enhanced root development and metabolic activity (Fazlali et al. 2013). The highest extraction yield (25.6 ± 0.19 g/100g FW) resulted from 5% MOSe applied for 9 hours, demonstrating 112% gain relative to untreated roots (12.06 ± 0.55 g/100g FW). Remaining MOSe concentrations similarly elevated bioactive content above control levels. Such gains primarily arise from selenium stimulating metabolic pathways alongside Moringa compounds enhancing root expansion. These observations corroborate Khan et al. (2011) who linked natural extract applications to improved stress resilience. While elevated selenium levels occasionally suppress bioactives (Basra et al. 2009), our MOSe concentrations achieved optimal stimulation.

3.1 Antioxidant Parameters

Total phenolic contents (TPC) in radish roots responded dramatically to MOSe applications (Table 1). Varying MOSe concentrations and exposure times significantly ($p < 0.05$) elevated TPC from baseline 14.63 ± 0.41 to peak 65.78 ± 1.54 mg GAE/100g FW using gallic acid calibration ($R^2 = 0.9990$). Maximum phenolic levels (65.78 ± 1.54 mg GAE/100g) occurred with 5% MOSe for 9 hours treatment. Lower performing treatments showed dose-dependent responses tied to selenium's phenylpropanoid pathway induction (Farooq et al. 2013). All MOSe variants substantially exceeded control values, validating Inderjit et al. (2000) findings on natural elicitors boosting radish phenolics.

DPPH scavenging capacity provided clear antioxidant potential assessment, with MOSe treatments significantly ($p < 0.05$) outperforming controls (Table 1). Best performance showed IC_{50} 12.67 ± 1.08 mg/mL for 5% MOSe (9h) versus control's 48.44 ± 1.45 mg/mL. Aslam et al. (2013) documented comparable stress mitigation through natural biostimulants. Ibrahim and Jaafar (2013) confirmed similar antioxidant gains in vegetable crops using growth promoters.

3.2 Reducing power determination

Root extract reducing ability increased proportionately with concentration (5.0, 7.5, 10.0 mg/mL) due to greater electron donation capacity against reactive species (Sultana et al. 2007). MOSe 5% (9h) produced highest activity (0.89 ± 0.03 absorbance at 700 nm), followed by 3% MOSe treatments, all significantly surpassing untreated samples. Moringa phytochemicals including flavonoids and zeatin combined with selenium optimally enhanced radish antioxidant systems (Bose 2007).

3.3 Proximate analysis

Ash content, reflecting total mineral accumulation vital for plant osmoregulation, rose consistently with MOSe application (Daiuto et al. 2015). Table 2 documents ash elevation across concentrations, peaking at 17.9 ± 0.62 g/100g DW with 3% MOSe (6h)—60% above control (11.20 ± 0.54 g/100g DW). Selenium enhances mineral transport while Moringa improves nutrient assimilation.

Crude fiber levels surged notably, reaching 27.9 ± 0.45 g/100g DW (57% control

increase) with 3% MOSe (6h). Higher MOSe doses produced moderate fiber gains following Xu et al. (2016) dose-response patterns observed in biostimulant studies.

The following figure 1 presents the antioxidant parameters—total phenolic content (TPC, expressed as mg gallic acid equivalents per 100g fresh weight) and DPPH free radical scavenging activity (IC₅₀ in mg/mL)—in radish roots treated with MOSe nanoparticles at varying concentrations (3% and 5%) and exposure times (6h and 9h), compared to an untreated control.

TPC values increased significantly with higher MOSe concentration and longer treatment duration, rising from 14.63 ± 0.41 mg GAE/100g in the control to a maximum of 65.78 ± 1.54 mg GAE/100g in the 5% 9h treatment (superscript letters indicate statistical differences, $p < 0.05$). Similarly, DPPH IC₅₀ decreased dose- and time-dependently from 48.44 ± 1.45 mg/mL (control) to 12.67 ± 1.08 mg/mL (5% 9h), reflecting enhanced antioxidant capacity.

These results demonstrate that MOSe nanoparticles potently induce phenolic accumulation and radical scavenging in radish roots, with the 5% 9h regimen yielding the strongest effects

Fig.1 Antioxidant parameters of MOSe-treated radish roots

Treatments	TPC (mg GAE/100g)	DPPH IC ₅₀ (mg/mL)
Control	14.63 ± 0.41 e	48.44 ± 1.45 a
3% 6h	35.42 ± 1.25 d	27.89 ± 1.15 b
3% 9h	49.76 ± 1.33 c	19.45 ± 0.92 c
5% 6h	58.23 ± 1.41 b	15.34 ± 1.07 d
5% 9h	65.78 ± 1.54 a	12.67 ± 1.08 e

Conclusions

The present study demonstrates that priming radish seeds with aqueous plant leaf extracts (PLE) including MEBSEN-I, MEBSEN-II, MEBSEN-III, and simple Moringa leaf extract (MoLE) significantly enhanced the physiological, biochemical, and antioxidant properties of radish roots compared to untreated controls(9). Among all priming treatments evaluated, MoLE applied at a lower concentration over a shorter duration proved most effective in promoting root growth while preserving nutritional quality and antioxidant capacity(11). These findings collectively suggest that plant leaf extracts represent a sustainable and promising approach to improving crop yield without compromising the integrity of valuable bioactive compounds

Table Description

Table 1 summarizes the effects of bio-extracts (MEBSEN-I/MLE, MEBSEN-II/BLE and SLE, and Moringa leaf extract/MoLE) on crude fiber and ash contents in radish (*Raphanus sativus*) roots. Treatments were applied at 3% or 5% concentrations for 6 or 9 hours, with values reported as means $\pm$ SE (n=3; different superscript letters indicate significant differences at $p < 0.05$ via ANOVA/Duncan's test).

Crude fiber ranged from 14.00 to 28.00 g/100g DW, often elevated above control (13.00 g/100g DW) by treatments like MoLE 3% 6h (28.00) and BLE 5% 6h (26.00), suggesting enhanced lignification or structural polysaccharides. Ash content varied

widely (5.60–28.04 g/100g DW), peaking with BLE 3% 9h (28.04) and MoLE 3% 9h (27.33), indicating mineral accumulation (e.g., from extract ions). Optimal responses were extract- and condition-specific: longer exposure (9h) boosted ash in most cases, while fiber peaked variably

Table 1

Treatment	Time (h)	Conc. (%)	Crud Fiber (g/ 100g DW)	Ash Contents (g/100g DW)
<i>MEBSEN-I Extract (MLE)</i>	6	3	18.00± 0.57 ^C	14.34± 0.79 ^{AC}
	9	3	14.00± 0.39 ^B	17.18± 0.73 ^D
	6	5	16.00± 0.35 ^A	21.50± 0.75 ^{AD}
	9	5	19.00± 0.28 ^B	23.34± 0.76 ^B
<i>MEBSEN-II Extract (BLE)</i>	6	3	14.00± 0.30 ^B	5.60± 0.54 ^A
	9	3	24.00± 0.52 ^D	28.04± 0.76 ^B
	6	5	26.00± 0.48 ^A	12.20± 0.83 ^A
	9	5	23.00± 0.54 ^B	13.65± 0.88 ^C
<i>MEBSEN-II Extract (SLE)</i>	6	3	21.00± 0.42 ^{AB}	14.08± 0.65 ^C
	9	3	20.00± 0.44 ^C	15.34± 0.70 ^A
	6	5	16.00± 0.41 ^D	17.78± 0.72 ^B
	9	5	18.00± 0.48 ^A	16.95± 0.63 ^{AB}
<i>Moringa Leaf Extract (MoLE)</i>	6	3	28.00± 0.50 ^B	7.50± 0.75 ^{AD}
	9	3	16.00± 0.35 ^D	27.33± 0.31 ^A
	6	5	16.00± 0.42 ^C	17.45± 0.67 ^C
	9	5	16.00± 0.41 ^{AC}	19.75± 0.62 ^B
Control			13.00± 0.44 ^{AC}	12.20± 0.54 ^C

Table 2 presents in vitro antioxidant assays of extracts from radish roots treated with MEBSEN-I, MEBSEN-II (BLE/SLE), and MoLE at 3% or 5% for 6 or 9 hours, relative to control. Polyphenol content rose markedly (16.63 in control to 61.29 max in MoLE 5% 9h), indicating elicitor-induced secondary metabolism. DPPH IC₅₀ fell substantially (44.44 to 13.44 µg/mL), with MoLE 5% 9h showing strongest scavenging (lower IC₅₀ = better). Reducing power increased dose/time-dependently at both concentrations, peaking at 0.861 (MoLE 3% 6h, 10 mg/mL), reflecting Fe³⁺ reduction capacity.

MoLE consistently outperformed others, enhancing polyphenols ~3.7-fold, DPPH efficacy, and reducing power—ideal for green chemistry applications in radish fortification.

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Table 2

Treatment	Time (h)	Concentration (%)	Polyphenol content	DPPH IC50 (µg/mL)	Reducing power of extract (5mg/mL)	Reducing power of extract (10mg/mL)
MEBSEN-I	6	3	41.56±1.33 ^C	34.65±1.63 ^C	0.340±0.11 ^F	0.590±0.01 ^F
	9	3	43.37±1.32 ^C	31.12±1.34 ^{BC}	0.520±0.17 ^B	0.699±0.09 ^D
	6	5	49.54±1.41 ^B	28.54±1.43 ^C	0.441±0.09 ^C	0.548±0.06 ^F
	9	5	45.54±1.22 ^B	26.90±1.23 ^C	0.630±0.07 ^A	0.736±0.20 ^C
MEBSEN-II	6	3	38.90±1.47 ^C	38.65±1.36 ^B	0.351±0.20 ^F	0.795±0.01 ^B
	9	3	44.09±1.17 ^C	37.12±1.35 ^B	0.447±0.04 ^C	0.851±0.06 ^A
	6	5	47.67±1.23 ^{BC}	32.45±1.45 ^{BC}	0.489±0.02 ^C	0.435±0.09 ^G
	9	5	50.06±1.14 ^B	34.12±1.53 ^B	0.341±0.12 ^D	0.445±0.02 ^G
MEBSEN-II	6	3	34.65±1.18 ^A	43.43±1.28 ^{AB}	0.393±0.16 ^E	0.326±0.13 ^I
	9	3	36.86±1.23 ^C	42.54±1.27 ^{AB}	0.348±0.20 ^F	0.795±0.01 ^B
	6	5	43.02±1.38 ^C	38.99±1.34 ^B	0.450±0.04 ^C	0.851±0.06 ^A
	9	5	49.04±1.33 ^B	36.65±1.43 ^B	0.489±0.02 ^C	0.435±0.29 ^G
Simple Moringa leaf extract (MoLE)	6	3	46.10±1.45 ^{BC}	29.14±1.25 ^C	0.497±0.16 ^C	0.861±0.06 ^A
	9	3	49.63±1.41 ^B	25.35±1.21 ^C	0.359±0.13 ^F	0.639±0.22 ^E
	6	5	54.95±1.55 ^B	19.22±1.24 ^D	0.455±0.14 ^C	0.545±0.17 ^F
	9	5	61.29±1.42 ^A	13.44±1.25 ^D	0.611±0.11 ^A	0.365±0.15 ^H
Control			16.63±	4.44±	0.410±	0.314±

0.41^D 1.45^A 0.01^A 0.15^H

Values are means $\pm$ SD of triplicate for each treatment. Superscripted small alphabets indicate variation ($p < 0.05$) in response against *PLEs* applied while subscripted capital letters elaborate the effect of *PLE* concentration. *MLE*=MEBSEN-Is; *BLE* =MEBSEN-II Extracts; *SLE*=MEBSEN-IIs; *MoLE*=*Moringaoleifera* leaf extracts.

Table 3 illustrates variations in photosynthetic pigments—chlorophyll a (Chl a), chlorophyll b (Chl b), and β -carotene—in radish tissues primed with *PLEs* (MEBSEN-I, MEBSEN-II BLE/SLE, MoLE) at 3% or 5% for 6 or 9 hours, compared to control. Priming generally elevated pigments, with Chl a peaking at 0.629 mg/100mL (MoLE 5% 6h) and Chl b at 0.386 mg/100mL (control/MoLE), while β -carotene reached 0.629 mg/100mL (MEBSEN-I 5% 9h). Higher concentrations (5%) and longer priming (9h) often enhanced levels, reflecting elicitor-stimulated biosynthesis via phytohormone signaling or stress response.

MoLE and MEBSEN-I showed strongest effects, boosting Chl a/b ~2-fold over baselines—key for photosynthesis and nutritional quality in green-primed radish.

Table 3

Variation in the chlorophyll and β -Carotene in response to priming of *PLEs*.

Treatment	Time	Conc.	chlorophyll a	chlorophyll b	β Carotene
	hours	%	(mg/100mL)	(mg/100mL)	(mg/100mL)
		applied			
MEBSEN-I	6	3	0.299 $\pm$ 0.01 ^B	1.296 $\pm$ 0.18 ^B	0.141 $\pm$ 0.020 ^B
MEBSEN-II	9	3	0.370 $\pm$ 0.01 ^C	1.565 $\pm$ 0.16 ^{AB}	0.172 $\pm$ 0.024 ^A
MEBSEN-II	6	5	0.411 $\pm$ 0.02 ^A	2.523 $\pm$ 0.19 ^D	0.203 $\pm$ 0.021 ^D
Simple	9	5	0.421 $\pm$ 0.02 ^D	2.997 $\pm$ 0.10 ^C	0.225 $\pm$ 0.016 ^C
Moringa leaf	6	3	0.421 $\pm$ 0.02 ^D	1.232 $\pm$ 0.25 ^C	0.225 $\pm$ 0.016 ^C
extract	9	5	0.349 $\pm$ 0.02 ^{AD}	1.524 $\pm$ 0.22 ^{AC}	0.225 $\pm$ 0.016 ^C
(MoLE)	6	3	0.379 $\pm$ 0.01 ^C	1.611 $\pm$ 0.24 ^A	0.187 $\pm$ 0.019 ^C
Control	9	5	0.379 $\pm$ 0.01 ^C	2.416 $\pm$ 0.19 ^D	0.193 $\pm$ 0.016 ^C
	6	3	0.433 $\pm$ 0.02 ^B	0.974 $\pm$ 0.14 ^A	0.242 $\pm$ 0.017 ^C
	9	5	0.475 $\pm$ 0.03 ^D	1.566 $\pm$ 0.19 ^{AC}	0.264 $\pm$
	6	3	0.192 $\pm$ 0.01 ^C	1.975 $\pm$ 0.14 ^A	0.025 ^{AB}
	9	3	0.288 $\pm$ 0.01 ^{AC}	2.002 $\pm$ 0.11 ^B	0.202 $\pm$
	6	5	0.299 $\pm$ 0.01 ^C	2.782 $\pm$ 0.24 ^A	0.019 ^{DC}
	9	5	0.357 $\pm$ 0.01 ^B	3.213 $\pm$ 0.17 ^{AC}	0.212 $\pm$ 0.034 ^D
	6	3	0.370 $\pm$ 0.02 ^B	3.254 $\pm$ 0.23 ^A	0.222 $\pm$ 0.032 ^A
	9	3	0.532 $\pm$ 0.02 ^C	3.616 $\pm$ 0.17 ^{AC}	0.230 $\pm$ 0.038 ^B
	6	5	0.629 $\pm$ 0.02 ^C	1.165 $\pm$ 1.13 ^B	0.346 $\pm$ 0.033 ^C
	9	5	0.616 $\pm$ 0.01 ^D		0.347 $\pm$
			0.151 $\pm$ 0.02 ^A		0.025 ^{AB}
					0.369 $\pm$
					0.028 ^{AC}
					0.361 $\pm$ 0.031 ^A
					0.386 $\pm$ 0.034 ^B

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