Nanobotanica
1(1): 2026



Research Article

ENHANCING MORPHOLOGICAL AND BIOCHEMICAL TRAITS OF *OCIMUM BASILICUM* L. THROUGH NANO SEED PRIMING

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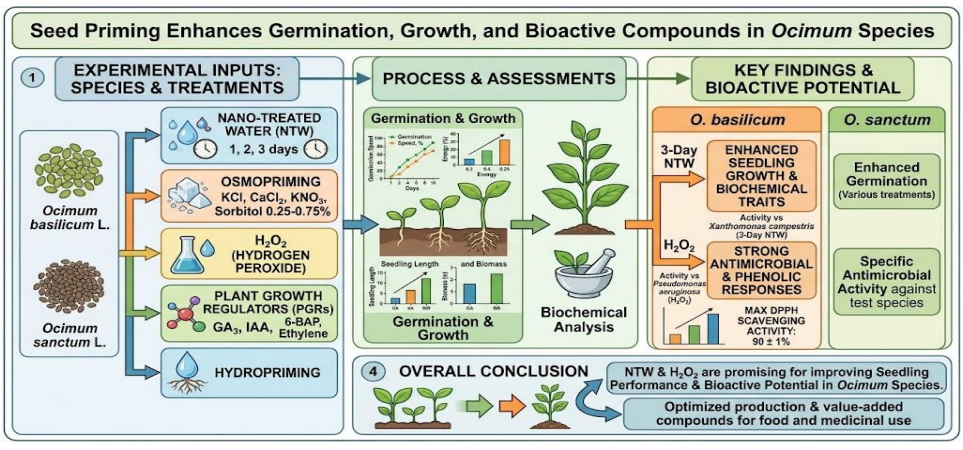
ABSTRACT

Ocimum basilicum L. is an economically important aromatic and medicinal plant used in food, cosmetics, and traditional medicine, but poor germination and seedling establishment can limit production. This study evaluated the effects of seed priming on germination, seedling growth, morphological traits, antimicrobial and antioxidant activities, and total phenolic content in *O. basilicum* and *O. sanctum*. Treatments included nano-treated water (NTW) for one, two, or three days; osmopriming with KCl, $CaCl_2$, KNO_3, and sorbitol (0.25–0.75%); H_2O_2; GA_3, IAA, 6-BAP, and ethylene; and hydropriming. Germination was assessed using germination speed, final germination percentage, and germination energy, while growth and yield-related traits were recorded at relevant developmental stages. Methanolic, petroleum ether, chloroform, and aqueous extracts were evaluated for antimicrobial activity using agar well diffusion. Antioxidant activity and total phenolic content were determined by DPPH radical-scavenging and Folin-Ciocalteu assays, respectively. Priming significantly influenced germination, growth, and biochemical responses in both species. Three-day NTW priming enhanced seedling growth and selected biochemical traits in *O. basilicum*, whereas H_2O_2 priming produced strong antimicrobial and phenolic responses. H_2O_2-primed samples showed activity against *Pseudomonas aeruginosa*, while three-day NTW-primed *O. basilicum* showed activity against *Xanthomonas campestris*. *O. basilicum* extracts exhibited up to 90 ± 1% DPPH radical-scavenging activity. Overall, NTW and H_2O_2 showed promise for improving seedling performance and bioactive potential in *Ocimum* species.

KEY WORDS: *Ocimum basilicum* L.; Seed nanopriming; Morphological traits; Biochemical traits; Antioxidant activity.

GRAPHICAL ABSTRACT:



Received: 20-04-2026, **Accepted**: 19-07-2026, **Published**: 15-08-2026
Citation: Younas A, Tanveer M, Khaliq S, Shahzadi Z, Riaz N, Rashid M, Maqbool Z. (2026). Enhancing morphological and biochemical traits of *Ocimum basilicum* L. through nano seed priming. Nanobotanica, 1(1), 71-91.

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1: INTRODUCTION

The genus *Ocimum*, belonging to the family Lamiaceae, comprises aromatic and medicinal plants widely used in food, cosmetics, and traditional medicine. Among these, *O. basilicum* and *O. sanctum* are economically important herbs valued for their culinary applications and bioactive constituents (Makri & Kintzios, 2008). The cultivation of *O. basilicum* across diverse regions reflects its importance as a source of fresh herbs and plant-derived products (Bączek *et al*., 2019). Improving crop establishment, productivity, and biochemical quality is therefore relevant to the cultivation and utilization of these species. Seed germination and seedling vigor are key determinants of successful crop establishment. Poor or uneven germination can result in inconsistent plant stands and limit subsequent growth. In basil, environmental stresses such as water deficit, salinity, and unfavorable temperatures can adversely affect germination and early seedling development (Thongtip *et al*., 2022). Pre-sowing treatments that improve seed performance may therefore support more uniform establishment and enhance subsequent plant development.

Seed priming involves controlled hydration that initiates metabolic processes before radicle emergence. Common approaches include hydropriming, osmo-priming, chemical priming, and hormonal priming. Depending on the treatment, seeds are exposed to water, osmotic solutions, chemical agents, or plant growth regulators. Treatment effectiveness depends on the species, priming agent, concentration, exposure duration, and growing conditions. Consequently, comparative evaluation is necessary to identify suitable treatments rather than assuming that a single priming method is equally effective across species and developmental stages. Previous research has examined priming effects on basil germination and early growth. Azarnia *et al*. (2016) investigated hydropriming, while Bojović *et al*. (2022) evaluated hormonal, halo-, osmo-, and hydropriming treatments in relation to germination and initial seedling development. These studies provide a foundation for investigating whether responses observed during germination extend to later growth and yield-related traits. Nano-treated water (NTW) represents an additional treatment requiring direct comparison with established approaches to determine its potential contribution to plant performance.

The value of *Ocimum* also depends on its biochemical properties. Javanmardi *et al*. (2003) reported variation in antioxidant activity and total phenolic content among *O. basilicum* accessions, highlighting the importance of assessing biochemical quality alongside agronomic traits. Antimicrobial activity provides another relevant measure of bioactive potential. Because extraction solvents influence the compounds recovered, evaluating extracts prepared using different solvents can provide a broader assessment of biological activity. Despite previous investigations of basil seed priming, further comparative evidence is needed to connect germination responses with subsequent morphological development, yield, and biochemical properties. Evaluating these outcomes within a common experimental framework would help clarify treatment-specific effects and identify approaches that offer benefits beyond early seedling establishment.

The present study compared NTW, osmotic, chemical, hormonal, and hydropriming treatments in *O. basilicum* and *O. sanctum*. The objectives were to (i) determine the effects of treatment

concentration and duration on germination and seedling performance; (ii) evaluate subsequent morphological and yield-related traits; and (iii) assess antimicrobial activity, DPPH radical-scavenging activity, and total phenolic content of extracts prepared using different solvents. The study aimed to identify promising priming treatments for improving agronomic performance and bioactive potential in the two species.

2: MATERIALS AND METHODS

2.1: Plant Material and Seed Priming Treatments

The study was conducted at the Botanical Garden and Molecular Taxonomy Laboratory, Department of Botany, Lahore College for Women University, Lahore, Pakistan, from April 2021 to August 2022. Seeds of *Ocimum basilicum* and *O. sanctum* were surface-sterilized with 3% sodium hypochlorite (NaClO) for 30 min and rinsed thoroughly with distilled water. Six seed-priming categories were evaluated: nano-treated water (NTW), osmo-priming, halopriming, chemical priming, hormonal priming, and hydropriming.

Osmo-priming treatments included KCl, $CaCl_2$, KNO_3, and sorbitol, while NaCl and H_2O_2 were used for halopriming and chemical priming, respectively. Hormonal treatments comprised GA_3, IAA, 6-BAP, and ethylene. Concentration-based treatments were applied at 0.25%, 0.50%, and 0.75%, whereas NTW and hydropriming were applied for one, two, or three days. Distilled water was used for hydropriming, and untreated seeds served as controls.

Each treatment was applied separately to both species in triplicate. NTW was prepared using a Qiangdi nano-863 treatment disc placed in a plastic container containing 20 L water, following the method of Younas *et al.* (2022) with modifications. Seeds were exposed to the respective priming treatments, shaken at 80 rpm for 2 h, and rinsed with distilled water before sowing.

2.2: In Vitro Seed Germination

Twenty seeds were placed in each sterile Petri dish, with three replicate dishes per treatment. The dishes were sealed with paraffin tape and incubated in darkness at 25 ± 2 °C. Germination was monitored, and speed of germination (SOG), final germination percentage (FGP), and germination energy percentage (GEP) were calculated.

Seedling fresh weight was recorded, followed by determination of dry weight after oven-drying at 50 °C for 24 h. Plant Cultivation and Morphological Measurements Germinated *O. basilicum* seedlings were transferred to soil and cultivated in the Botanical Garden. Three biological replicates were maintained for each treatment. Morphological traits were recorded during early vegetative growth and at 14 weeks. Measurements included length, width, and weight of whole plants, roots, stems, and leaves, as well as leaf number and inflorescence length, width, weight, and number, where applicable, following the general methodology of Aflatooni *et al.* (2019).

2.3: Plant Extracts and Bioactivity Assays

Methanolic, petroleum ether, chloroform, and aqueous extracts of *O. basilicum* were evaluated for antimicrobial activity using the agar well-diffusion method of Balouiri *et al.* (2016). The test organisms included *Pseudomonas aeruginosa*, *Xanthomonas campestris* pv. *hederae*, *Neurospora crassa*, and *Aspergillus niger*. Cefuroxime and fluconazole (150 mg/10 mL DMSO) served as positive controls for bacterial and fungal assays, respectively. Plates were incubated at 37 °C for bacteria and 28 °C for fungi, and inhibition zones were measured after 48 h.

Antioxidant activity was determined using the DPPH radical-scavenging assay following Blois (1958). Extracts were mixed with 0.1 mM DPPH and incubated in darkness for 30 min, after which absorbance was measured at 517 nm. Radical-scavenging activity was calculated as

$$= [(A_{control} - A_{sample})/A_{control}] \times 100.$$

Total phenolic content was determined using the Folin-Ciocalteu method. Extracts were reacted with diluted Folin-Ciocalteu reagent and 7.5% sodium carbonate, incubated for 30 min, and measured at 725 nm. Results were expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g).

2.4: Statistical Analysis

Data was analyzed using CoStat (CS6204) and SPSS 20.0. All treatments were replicated three times. Analysis of variance (ANOVA) was performed, with two-way or three-way ANOVA used where appropriate to assess main effects and interactions. Treatment means were compared using Duncan's multiple range test or least significant difference test at $P \leq 0.05$. Different lowercase letters indicated significant differences among relevant treatment means.

3: RESULTS AND DISCUSSION

3.1: Germination Parameters

Seed priming responses in *O. basilicum* varied among treatments and treatment levels (Table 1). The highest speed of germination (SOG) was recorded with H_2O_2 at 0.75% (15.8 ± 0.1), compared with 4.25 ± 0.2 in the unprimed control. Among osmopriming treatments, sorbitol at 0.25% produced the highest SOG (13.1 ± 0.1), followed by $CaCl_2$ at 0.25% (12.4 ± 0.3).

Three-day NTW treatment yielded an SOG of 12.6 ± 0.1, while IAA at 0.50% produced the highest value among the unambiguously listed hormonal treatments (12.1 ± 0.1). The highest final germination percentage (FGP; 90 ± 5%) was observed with sorbitol and $CaCl_2$ at 0.25%, H_2O_2 at 0.75%, and three-day NTW treatment, compared with 35 ± 5% in the control. Germination energy percentage (GEP) was highest with H_2O_2 at 0.25% (70 ± 5%), followed by H_2O_2 at 0.75% (65 ± 5%) and sorbitol at 0.25% (60 ± 5%).

Three-day NTW treatment produced a GEP of 50 ± 5%, compared with 15 ± 5% in the control. Increasing treatment concentration did not consistently improve germination, indicating that responses depended on the priming agent and treatment level.Seed priming involves controlled hydration that initiates pre germinative metabolic processes before radicle emergence. These processes can include cellular repair and antioxidant responses that support germination and seedling establishment (Paparella *et al*., 2015).

The favorable germination responses following H_2O_2 treatment may be associated with its role in redox signaling. H_2O_2 is a reactive oxygen species that can act as a signaling molecule and influence antioxidant defense, although its effects depend on concentration and exposure conditions (Fujita & Hasanuzzaman, 2022).

3.2: Morphological Characteristics during Early Vegetative Growth

Morphological traits of *O. basilicum* varied among priming treatments and concentrations (Table 2). The highest whole-plant length was recorded with KNO_3 and IAA at 0.25% (18 ± 1), compared with 12 ± 1 in the unprimed control.

$CaCl_2$ at 0.25% produced the highest whole-plant weight (16 ± 0.5), root length (5.5 ± 0.3), stem length (8 ± 1), and stem width (6.5 ± 1). The corresponding control values were 9 ± 1, 2.1 ± 0.1, 2.1 ± 1, and 2 ± 1, respectively. Ethylene at 0.75% produced the highest leaf length (4 ± 0.1), width (3 ± 0.1), and weight (4 ± 1).

The highest leaf number (10 ± 1) was recorded with KCl at 0.25% and GA_3 at 0.75%, compared with 3 ± 1 in the control. Initial growth began in week 2 under several treatments, whereas the control began growth in week 4.

However, numerical differences should be distinguished from statistically supported differences. These measurements describe plant size and biomass; they do not independently establish increased seed yield or stress tolerance.

Previous work on basil priming also emphasizes germination and early growth responses (Bojović *et al*., 2022).

3.3: Morphological Traits at Week 14

Morphological traits of primed *O. basilicum* plants differed among treatments at week 14 (Table 3). H_2O_2 priming at 0.25% produced a root length of 32 ± 2, compared with 25 ± 1 in the unprimed control, whereas one-day NTW treatment produced a root length of 22 ± 2. Leaf length following GA_3 priming at 0.25% was 30 ± 3, compared with 15 ± 3 in the control.

One-day NTW treatment produced a leaf length of 15 ± 3, equal to the control mean. Variation was also observed in root width and weight, leaf width and weight, and leaf number. ANOVA results are presented in table 4& 5.

3.4: Antibacterial Activity

Antibacterial activity varies with the priming treatment and extraction solvent (Fig. 2a, 2b, 2c, 2d, and 2e).

Against *Xanthomonas campestris*, the petroleum ether extract of IAA-primed *O. basilicum* at 0.50% produced an inhibition zone of 86 ± 1 mm, compared with 43 ± 0.5 mm for the cefuroxime positive control. Methanolic extracts of three-day NTW-treated *O. basilicum* produced an inhibition zone of 83.8 ± 1 mm, while petroleum ether extracts of NTW-treated plants yielded 82.2 ± 1 mm.

The chloroform extract of ethylene-primed *O. sanctum* at 0.50% produced an inhibition zone of 32 ± 0.5 mm. Against *Pseudomonas aeruginosa*, the largest inhibition zone among the values described was recorded for the aqueous extract of GA_3 -primed *O. basilicum* at 0.50% (88 ± 1 mm).

Aqueous and petroleum ether extracts of $CaCl_2$ -primed plants at 0.25% produced inhibition zones of 86 ± 1 and 84 ± 0.5 mm, respectively.

Smaller zones were recorded for the chloroform extract of sorbitol-primed plants at 0.50% (35 ± 0.2 mm) and the petroleum ether extract of 6-BAP-primed plants at 0.25% (31 ± 0.2 mm).

These observations indicate that antibacterial activity differed among combinations of priming treatment and extraction solvent.

Differences among solvents may reflect variation in the compounds recovered during extraction. Solvent-dependent phenolic recovery and biological activity have also been reported in medicinal plant extracts (Mehmood *et al*., 2022).

Table 1. Effects of seed priming treatments on germination parameters of *O. basilicum* L.

Osmo-priming	Conc. (%)	SOG (%)	FGP (%)	GEP (%)	Hormonal-priming	Conc. (%)	SOG (%)	FGP (%)	GEP (%)
Sorbitol ($C_6H_{14}O_6$)	**0.25**	13.1±0.1^{b}	90±5^{c}	60±5^{a}	**Auxin (IAA)**	**0.25**	6.1±0.05^{a}	55±5^{a}	25±0.5^{a}
	0.5	7.7±0.3^{a}	60±5^{c}	30±5^{a}		**0.5**	12.1±0.1^{b}	85±5^{a}	45±5^{a}
	0.75	5.8±0.2^{a}	60±5^{c}	30±5^{a}		**0.75**	6.1±0.3^{a}	60±5^{b}	20±5^{b}
Potassium nitrate (KNO_3)	**0.25**	9.7±0.3^{a}	70±5^{d}	35±5^{a}	**Gibberellic acid (GA_3)**	**0.25**	7.7±0.2^{a}	50±5^{a}	30±5^{a}
	0.5	6.3±0.4^{a}	55±5^{ab}	25±5^{a}		**0.5**	7.5±0.3^{bc}	70±5^{a}	35±5^{a}
	0.75	10.8±0.1^{b}	75±5^{a}	45±5^{b}		**0.75**	9.6±0.2^{ab}	85±5^{a}	35±5^{b}
Potassium chloride (KCL)	**0.25**	11.3±0.1^{b}	80±5^{a}	30±5^{b}	**Cytokinin (6-BAP)**	**0.25**	4.7±0.2^{a}	50±5^{bc}	15±5^{bc}
	0.5	9.6±0.07^{b}	65±5^{a}	40±5^{b}		**0.5**	7.8±0.3^{c}	70±5^{c}	40±5^{c}
	0.75	8.7±0.2^{ac}	55±5^{b}	35±5^{c}		**0.75**	7.1±0.1^{a}	55±5^{a}	30±5^{ab}
Calcium chloride ($CaCl_2$)	**0.25**	12.4±0.3^{b}	90±5^{b}	35±5^{c}	**Ethylene (C_2H_4)**	**0.25**	4.3±0.1^{a}	35±5^{a}	15±5^{b}
	0.5	9.2±0.07^{a}	65±5^{b}	35±5^{c}		**0.5**	3.7±0.3^{ab}	35±5^{ab}	10±5^{b}
	0.75	6.1±0.2^{a}	55±5^{c}	26±5^{a}		**0.75**	9.8±0.2^{bc}	60±5^{bc}	40±5^{a}
Control	***0***	4.25±0.2^{b}	35±5^{ab}	15±5^{a}	**Control**	***0***	4.25±0.2^{b}	35±5^{b}	15±5^{b}
Hydro-priming	**1 day**	6.5±0.06^{a}	45±5^{b}	20±5^{b}	**Chemical-priming (H_2O_2)**	**0.25**	13.0±0.1^{c}	85±5^{c}	70±5^{c}
	2 day	9.1±0.4^{b}	70±5^{a}	35±5		**0.5**	14.0±0.2^{a}	75±5^{a}	55±5^{a}
	3 day	9.5±0.2^{b}	75±5^{b}	35±5^{a}		**0.75**	15.8±0.1^{a}	90±5^{a}	65±5^{a}
Halo-priming (Sodium chloride NaCl)	**0.25**	4.7±0.3^{ab}	40±5^{ab}	15±5^{ab}	**Ethylene (C_2H_4)**	**0.25**	6.5±0.06^{a}	45±5^{b}	20±5^{b}
	0.5	10.4±0.2^{bc}	65±5^{bc}	40±5^{bc}		**0.5**	9.1±0.4^{b}	70±5^{a}	35±5
	0.75	6.7±0.2^{a}	45±5^{a}	25±5^{a}		**0.75**	9.5±0.2^{b}	75±5^{b}	35±5^{a}
Nano-treated water	**1 day**	5.6±0.07^{a}	40±5^{a}	25±5^{b}	**Control**	***0***	4.25±0.2^{b}	35±5^{b}	15±5^{b}
	2 day	6.5±0.1^{a}	45±5^{a}	25±5^{a}					
	3 day	12.6±0.1^{a}	90±5^{a}	50±5^{a}					

Values represent mean ± SD of three replicates. SOG, speed of germination; FGP, final germination percentage; GEP, germination energy percentage; NTW, nano-treated water. Within each statistical comparison, means sharing at least one lowercase superscript letter do not differ significantly, whereas means with no common letter differ significantly at $P \leq 0.05$. The control consisted of unprimed seeds. NTW and hydropriming treatment durations are expressed in days.

Table 2. Morphological traits of *O. basilicum* during early vegetative growth following osmotic and hormonal seed priming.

Priming treatment	Optimum concentration (%)	Plant length	Root length	Stem length	Leaf number
Sorbitol	0.75	17 ± 1	4.2 ± 0.1	6.7 ± 1	3 ± 1
KCl	0.25	10 ± 0.1	3.3 ± 1	6 ± 1	10 ± 1
KNO_3	0.25	18 ± 1	2.5 ± 0.1	5.5 ± 1	8 ± 1
$CaCl_2$	0.25	15 ± 0.5	5.5 ± 0.3	8 ± 1	9 ± 1
IAA	0.25	18 ± 1	2.5 ± 0.1	5.5 ± 1	8 ± 1
GA_3	0.25	11 ± 0.2	4.5 ± 0.1	7 ± 1	8 ± 1
6-BAP	0.50	9.2 ± 0.1	3.5 ± 1	6.2 ± 1	7 ± 1
Ethylene	0.75	10 ± 1	5 ± 0.1	7.6 ± 1	8 ± 1
Control	—	12 ± 1	2.1 ± 0.1	2.1 ± 1	3 ± 1

Values represent mean ± SD of three replicates. Conc., concentration; IAA, indole-3-acetic acid; GA_3, gibberellic acid; 6-BAP, 6-benzylaminopurine. Within each statistical comparison, means sharing at least one lowercase superscript letter do not differ significantly, whereas means with no common letter differ significantly at $P \leq 0.05$. The control consisted of unprimed seeds.

Table 3. The most effects of seed priming treatments on morphological traits of *O. basilicum* at week 14.

Plant part	Best priming treatment	Length	Fresh weight	Number
Whole plant	NTW, 3 days	90 ± 1	509 ± 1	—
Root	NTW, 3 days	32 ± 2	22 ± 2	—
Stem	NTW, 3 days	58 ± 1	182 ± 1	—
Leaves	H_2O_2, 0.50%	21 ± 3	13 ± 3	381 ± 1
Inflorescence	NTW, 3 days	35 ± 2	29 ± 2	69 ± 2
Control	Unprimed	76 ± 1	406 ± 1	—

Values represent mean ± SD of three replicates. Conc., concentration; NTW, nano-treated water; IAA, indole-3-acetic acid; GA_3, gibberellic acid; 6-BAP, 6-benzylaminopurine. A dash indicates that the entry is not applicable. Within each statistical comparison, means sharing at least one lowercase superscript letter do not differ significantly, whereas means with no common letter differ significantly at $P \leq 0.05$. The control consisted of unprimed seeds.

Table 4. Three-way ANOVA summary for germination and morphological traits assessed during early vegetative growth and at week 14

(1) Germination				(2) Morphology					
Treatments	**DF**	**F value**	**Sign. value**	**Initial**			**Final**		
				DF	**F value**	**Sign. value**	**DF**	**F value**	**Signif.**
Factor (A)	1	16.76	***	1	27.67	***	1	30.76	***
Priming treatment (B)	5	80.95	***	5	92.95	***	5	83.5	***
Concentration (C)	2	17.27	**	2	24.27	***	2	28.27	**
A × B	5	27.01	*	5	30.01	**	5	32.01	**
A × C	2	6.93	*	2	8.93	**	2	9.93	**
B × C	10	0.73	*	10	0.63	**	10	0.93	**
A × B × C	10	7.406	**	10	8.406	**	10	9.406	*

DF, degrees of freedom; ×, interaction between factors. Initial and final refer to early vegetative growth and week 14, respectively. Significance levels: *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

Table 5. Three-way ANOVA summary for antibacterial activity, antifungal activity, DPPH radical-scavenging activity, and total phenolic content

(1) Antimicrobial activity							(2) Antioxidant activity					
Antifungal				**Antibacterial**			**DPPH assay**			**TPC assay**		
Treatments	**DF**	**F value**	**Sign.**	**DF**	**F value**	**Sign.**	**DF**	**F value**	**Sign.**	**DF**	**F value**	**Sign.**
Factor (A)	1	108.4	***	1	112.5	***	1	108.4	***	1	108.4	***
Priming treatment (B)	5	13.2	*	5	0.500	**	5	56.0	**	5	64.05	***
Concentration (C)	2	50.2	***	2	47.844	***	2	9.87	***	2	42.7	*
A × B	5	118.2	***	5	120.12	***	5	137.2	***	5	167.2	***
A × C	2	5.855	*	2	6.844	*	2	10.855	**	2	15.855	*
B × C	10	6.348	**	10	8.469	**	10	16.267	*	10	.348	**
A × B × C	10	5.51	**	10	6.969	*	10	8.51	*	10	9.51	*

DF, degrees of freedom; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TPC, total phenolic content. A, [actual factor name]; B, priming treatment; C, concentration. × indicates an interaction between factors. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

3.5: Antifungal Activity

Antifungal activity of *Ocimum basilicum* extracts prepared using different solvents was evaluated against *Aspergillus niger* and *Neurospora crassa* using the agar well-diffusion method. Activity was expressed as inhibition-zone diameter and compared with fluconazole (Fig. 3a, 3b, 3c, and 3d), which produced a zone of 25 ± 0.5 mm. Inhibition varied with the priming treatment, treatment level, extraction solvent, and test organism.

Against *A. niger*, the largest reported inhibition zone was produced by the aqueous extract of H_2O_2-primed plants at 0.75% (86 ± 1 mm), followed by the aqueous extract of GA_3-primed plants at 0.50% (79 ± 1 mm) and the chloroform extract of KCl-primed plants at 0.25% (77 ± 1 mm). Three-day NTW treatment produced an inhibition zone of 68 ± 0.5 mm. Smaller zones were recorded for the petroleum ether extract following one-day NTW treatment (21 ± 0.5 mm) and the chloroform extract following three-day hydropriming (24 ± 0.5 mm).

Against *N. crassa*, the largest reported inhibition zone was recorded for the chloroform extract of GA_3-primed plants at 0.50% (86 ± 1 mm), followed by the chloroform extract of H_2O_2-primed plants at 0.75% (82 ± 1 mm). The petroleum ether extract of $CaCl_2$-primed plants at 0.50% produced an inhibition zone of 66 ± 1 mm. Methanolic extracts following three-day NTW treatment and chloroform extracts following one-day hydropriming produced zones of 55 ± 1 and 52 ± 0.1 mm, respectively.

Unprimed extracts also exhibited antifungal activity. Against *A. niger*, inhibition zones ranged from 19 ± 0.5 mm for the aqueous extract to 24 ± 0.2 mm for the petroleum ether extract. Against *N. crassa*, the highest reported activity among unprimed extracts was observed in the aqueous extract (34 ± 0.1 mm). Overall, selected priming treatments produced larger inhibition zones than those reported for unprimed extracts, although the response depended on the treatment, solvent, and fungal species.

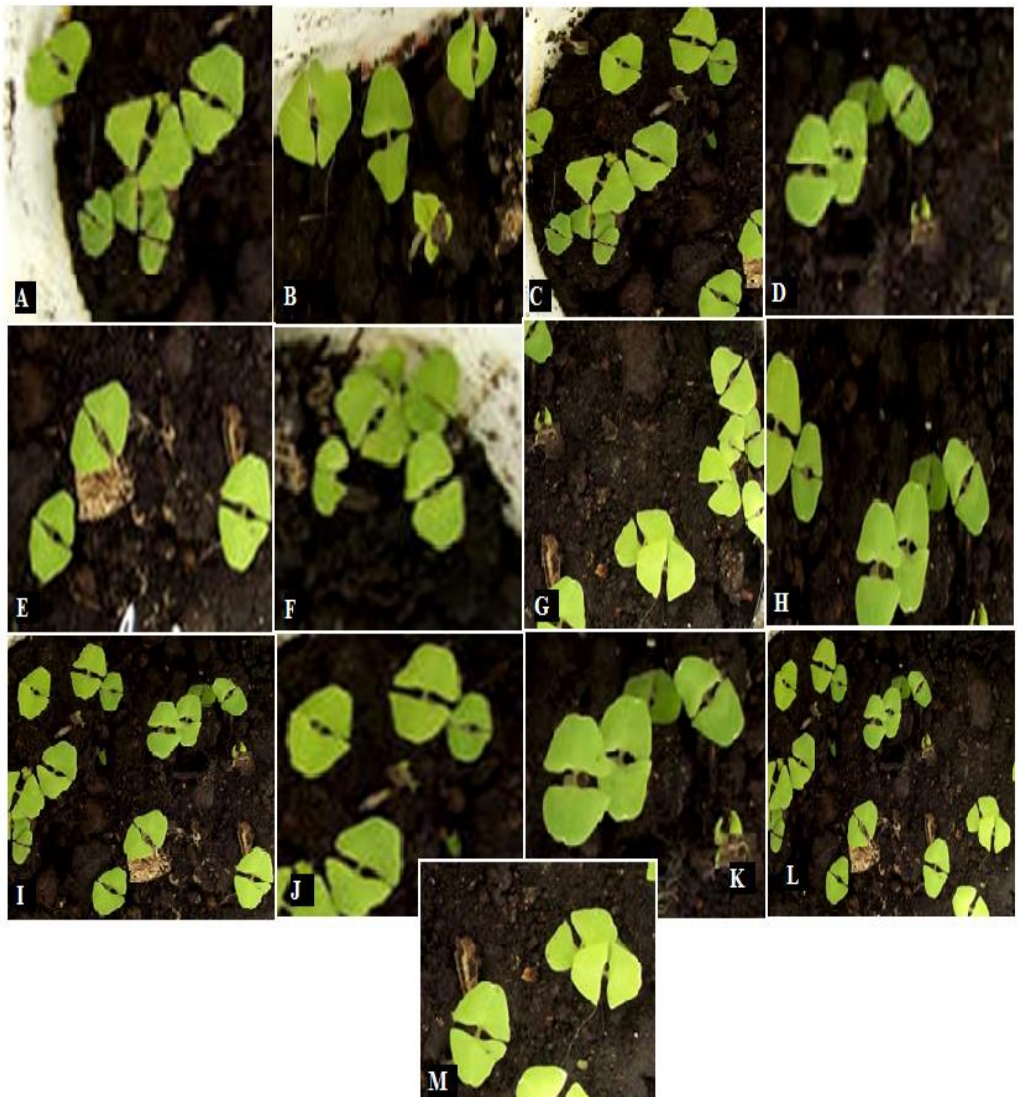

Figure 1. Morphological changes of *O. basilicum* seedlings after the seeds are primed for early vegetative growth stages. (A) Sorbitol; (B) KNO_3; (C) $CaCl_2$; (D) KCl; (E) ethylene; (F) 6-BAP; (G) GA_3; (H) IAA; (I) H_2O_2; (J) NaCl; (K) hydro-priming; (L) nano-priming; and (M) non-primed control.

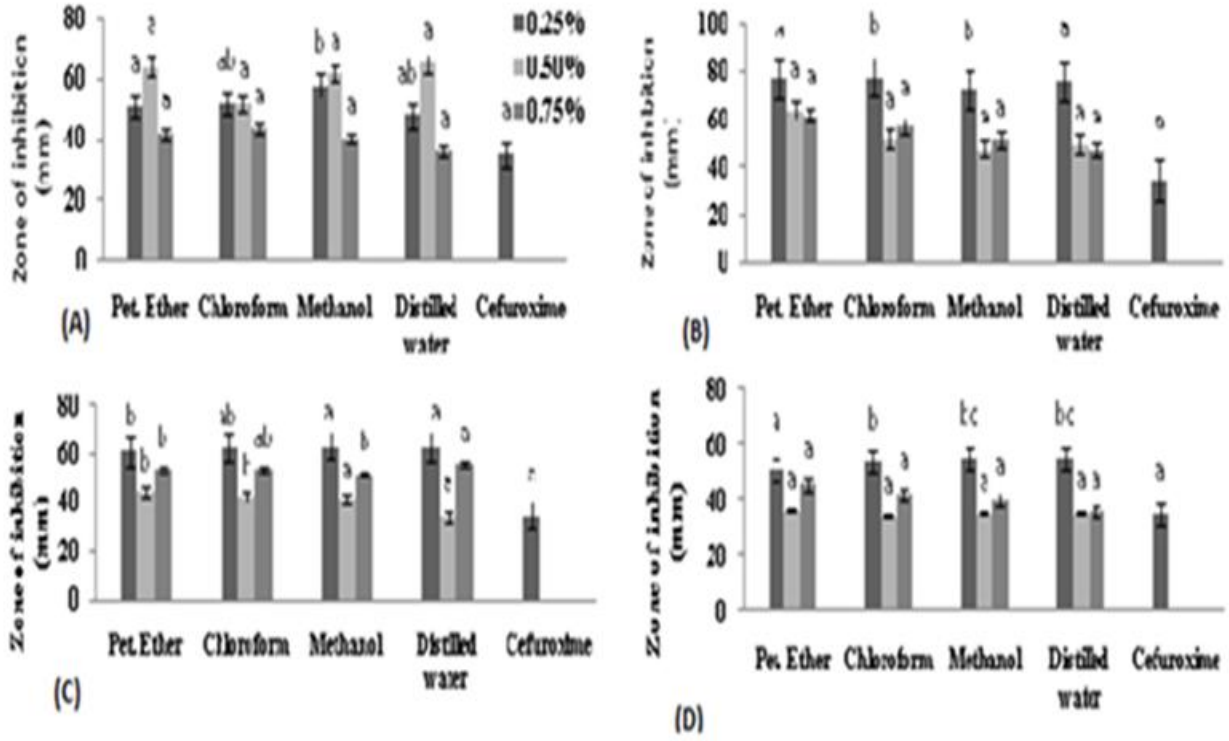

Figure 2a: The antibacterial activity of osmotic-primed extracts of *O. basilicum* seeds against *P. aeruginosa*. (A) $CaCl_2$; (B) KCl; (C) KNO_3; and (D) sorbitol

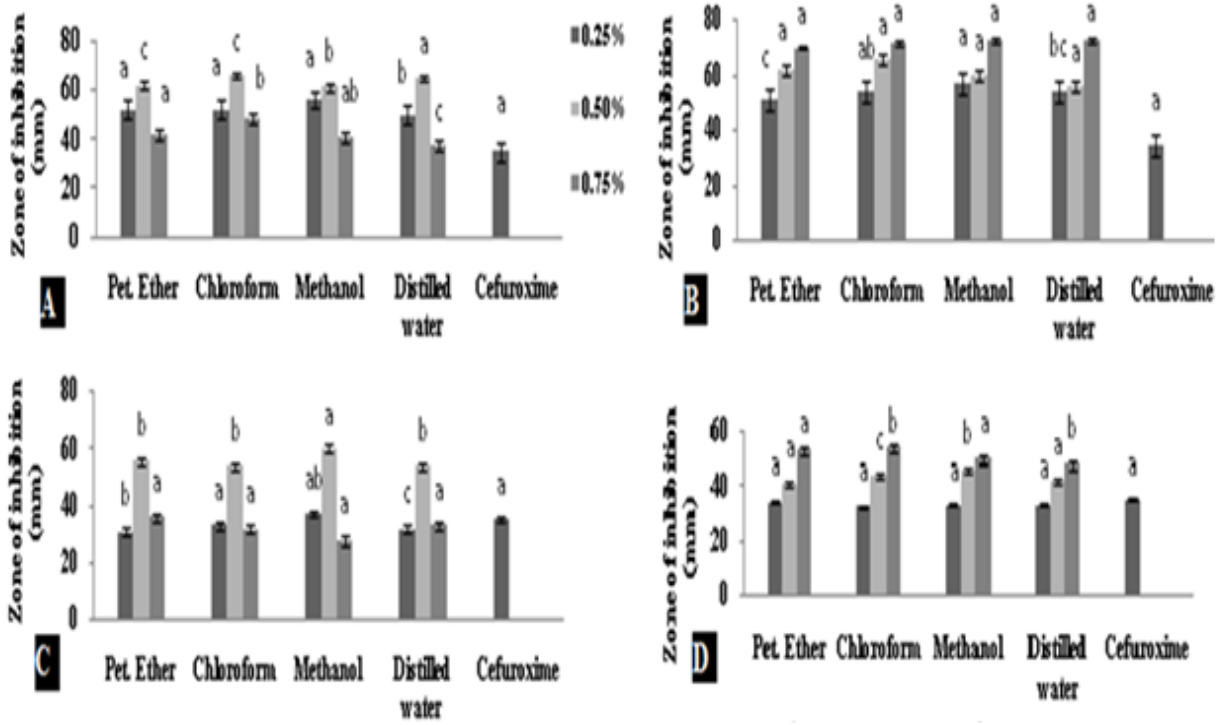

Figure 2b. The antibacterial activity of osmotic-primed extracts of *O. basilicum* seeds against *P. aeruginosa* (A) GA_3; (B) IAA; (C) 6-BAP; and (D) ethylene.

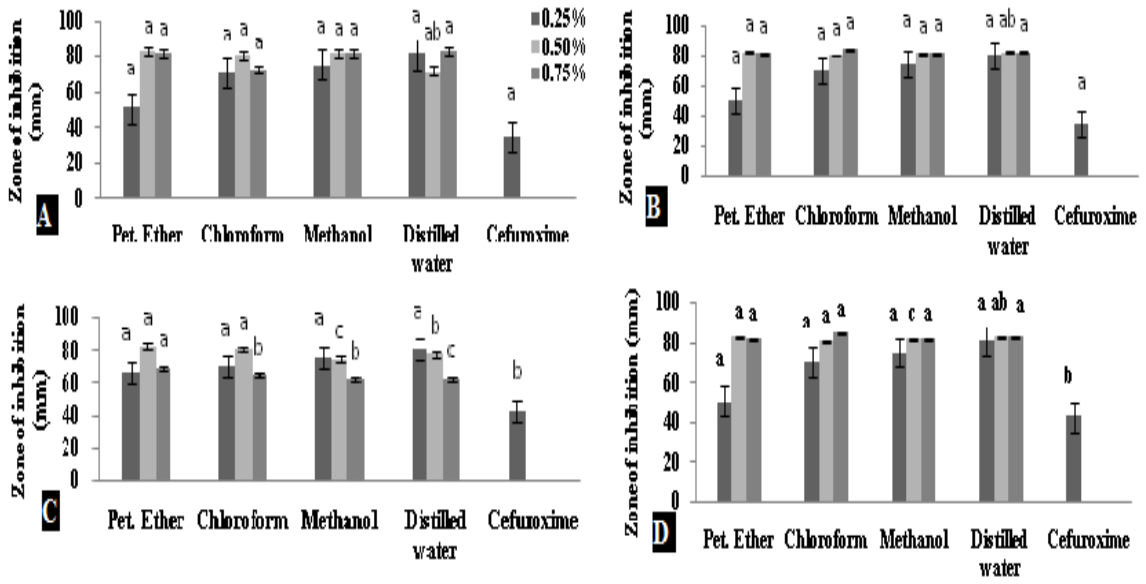

Figure 2c. Anti-bacterial property of extracts from H_2O_2 treated seeds against bacterial pathogens. (A) *O. sanctum* against *P. aeruginosa*; (B) *O. sanctum* against *X. campestris*; (C) *O. basilicum* against *P. aeruginosa*; and (D) *O. basilicum* against *X. campestris*

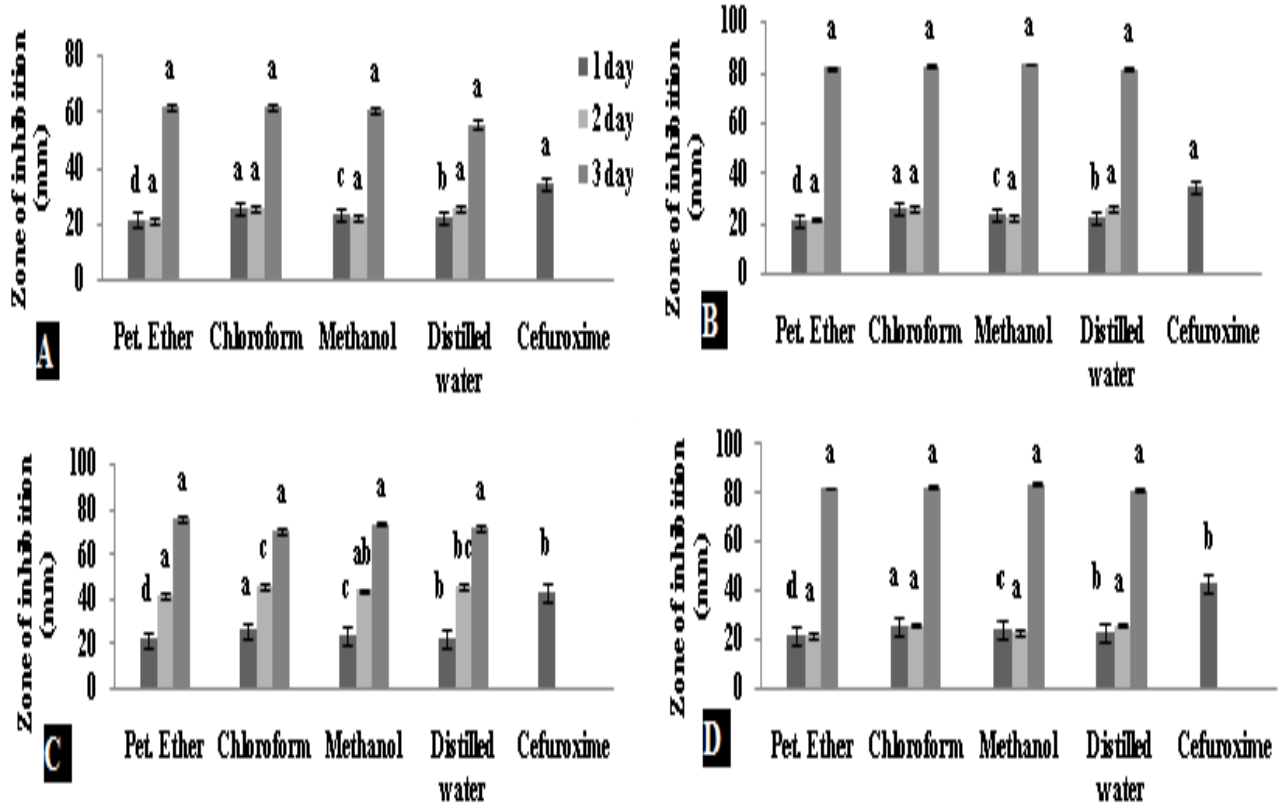

Figure 2d. Antibacterial activity of nano-treated extracts of seeds against the bacterial pathogens after various duration of treatment. A) *O. sanctum* + *P. aeruginosa*; B) *O. sanctum* + *X. campestris*; C) *O. basilicum* + *P. aeruginosa*; and D) *O. basilicum* + *X. campestris*

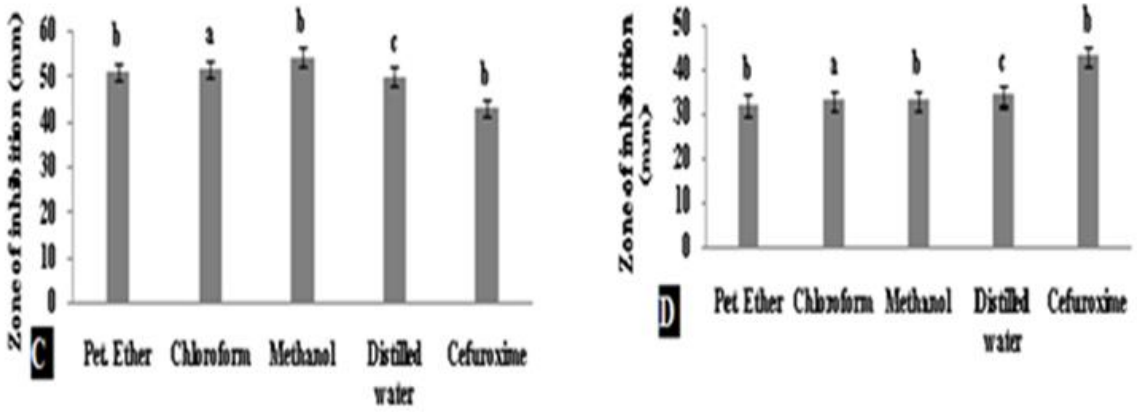

Figure 2e: Antibacterial effect of without primed *Ocimum basilicum* seed under variable extracts (C) *O. basilicum* with *Pseudomonas aeruginosa* (D) *O. basilicum with Xanthomonas campestris*

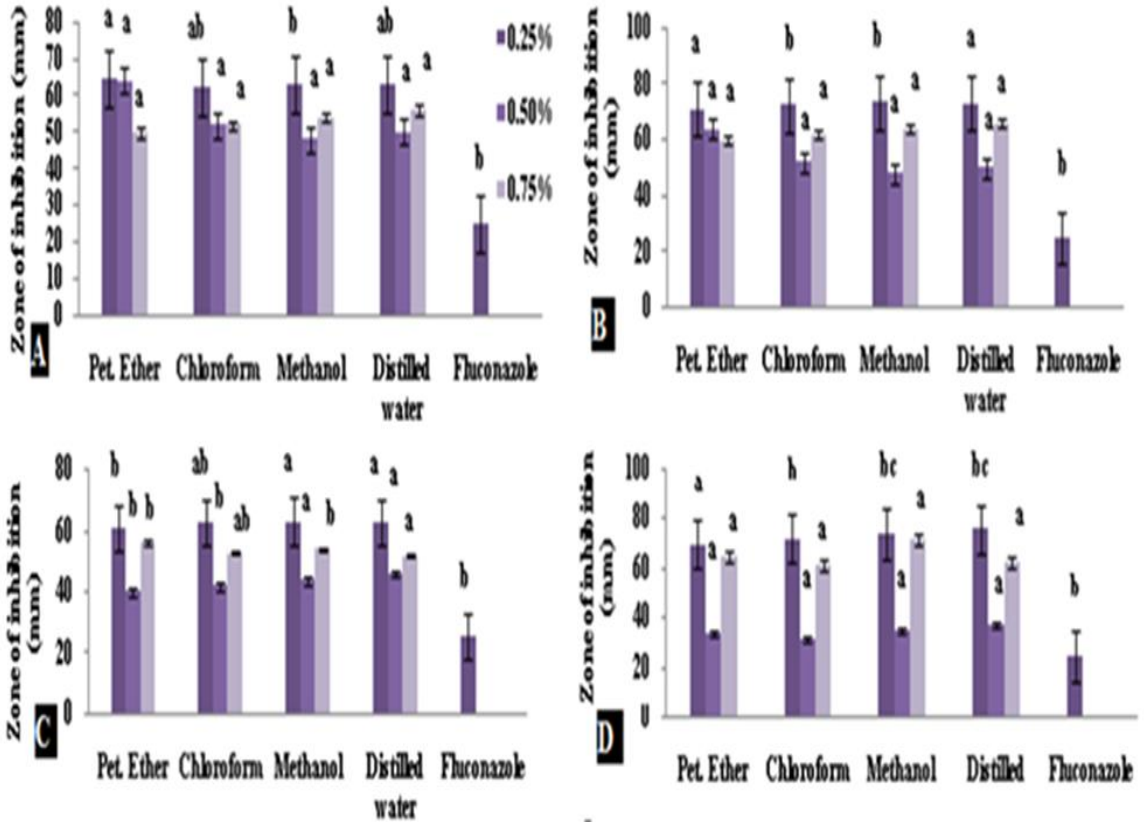

Fig 3a: Antifungal effect under three diff. conc. of osmo-primed *Ocimum basilicum* seed extracts (A) Calcium chloride with *Aspergillus niger* (B) Potassium chloride with *Aspergillus niger* (C) Potassium nitrate with *Aspergillus niger* (D) Sorbitol with *Aspergillus niger*

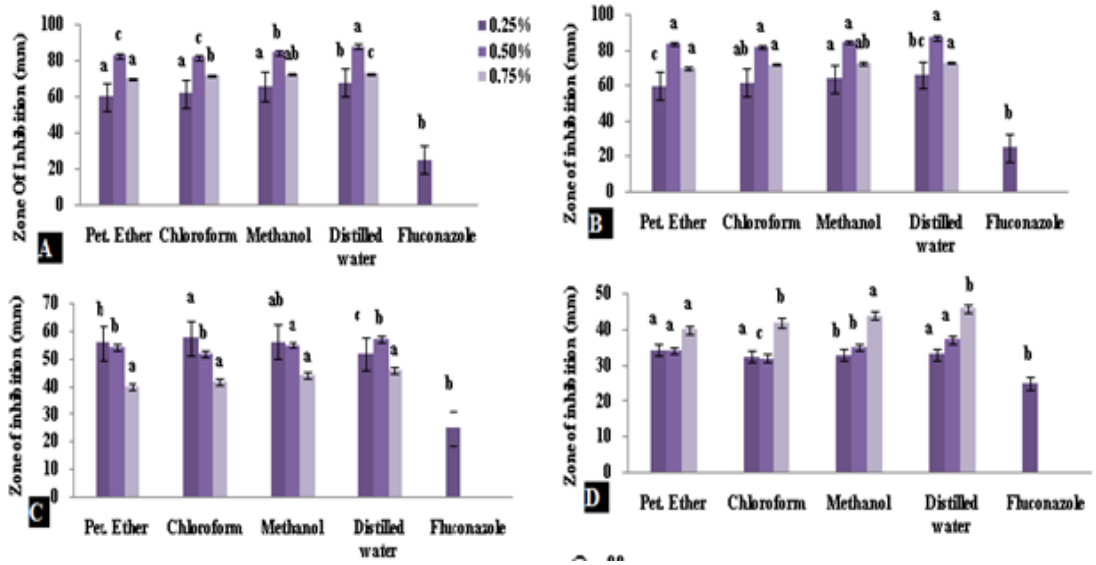

Fig 3b: Antifungal effect under three diff. conc. of hormonal-primed *Ocimum sanctum* and *Ocimum basilicum* seed extracts (A) Gibberellic acid with *Aspergillus niger* (B) Auxin with *Aspergillus niger* (C) Cytokinin with *Aspergillus niger* (D) Ethylene with *Aspergillus niger*

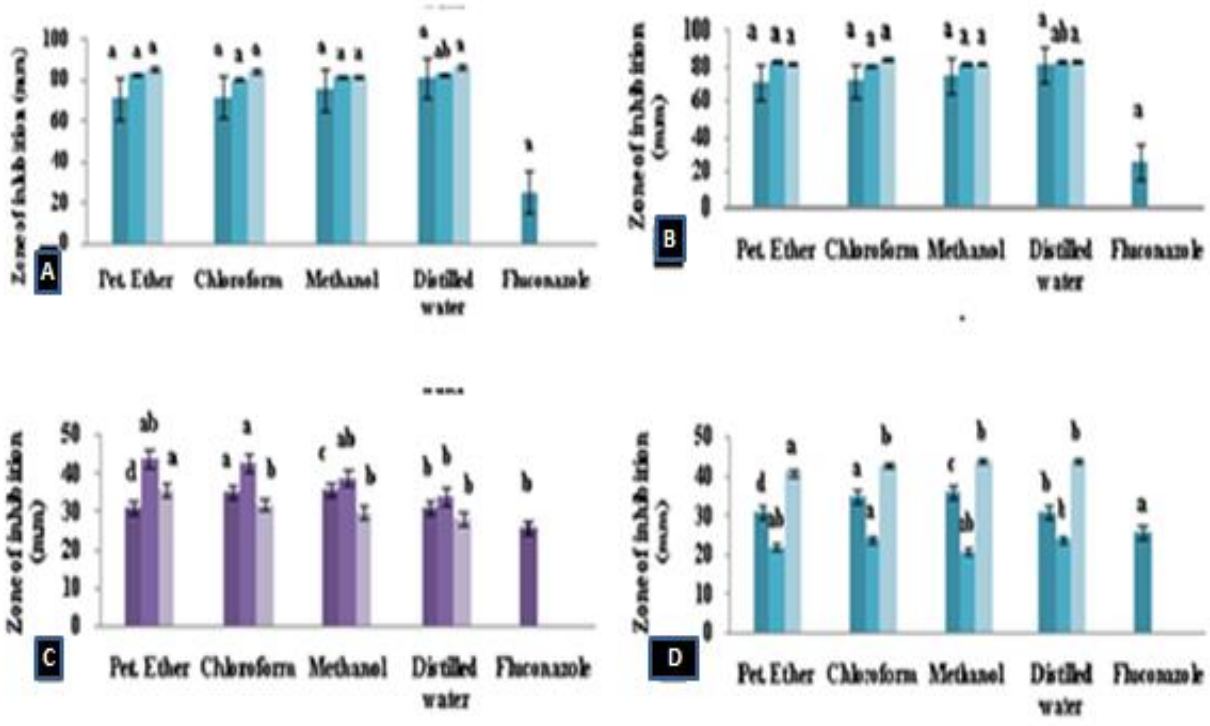

Fig 3c: Antifungal effect under three diff. conc. of chemical and halo-primed *Ocimum basilicum* seed extracts (A) Hydrogen peroxide primed *O. basilicum* with *Aspergillus niger* (B) Hydrogen peroxide primed *O. basilicum* with *Neurospora crassa* (C) Sodium chloride-primed *O. basilicum* with *Aspergillus niger* (D) Sodium chloride-primed *O. basilicum* with *Neurospora crassa*

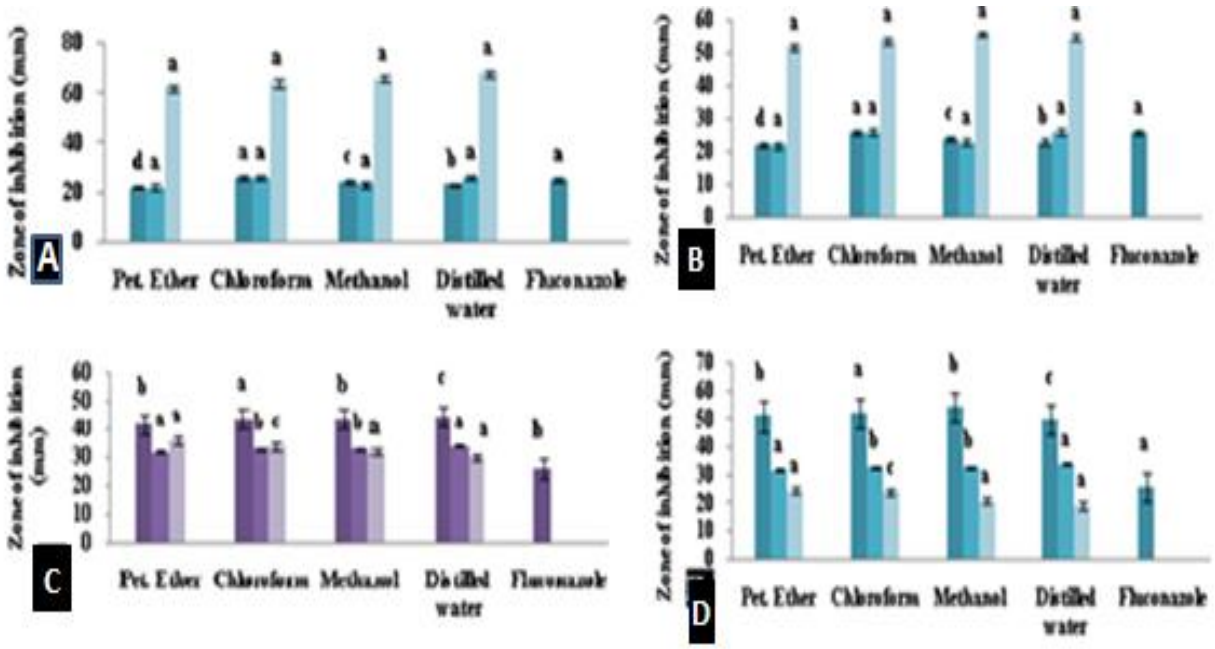

Fig 3d: Antifungal effect under three different days of nano and hydro-primed and variable extract *Ocimum basilicum* seed extracts (A) Nano-primed *O. basilicum* with *Aspergillus niger* (B) Nano-primed *O. basilicum* with *Neurospora crassa* (C) Hydro-primed *O. basilicum* with *Aspergillus niger* (D) Hydro-primed *O. basilicum* with *Neurospora*

3.6: Antioxidant activity DPPH Assay

Antioxidant activity of *O. basilicum* extracts varied among priming treatments and extraction solvents (Fig. 4a, 4b, 4c, 4d, and 4e). The highest reported DPPH radical-scavenging activity was recorded for the aqueous extract following three-day NTW treatment (96 ± 1%).

High activity was also reported for the chloroform extracts following three-day hydropriming and NaCl priming at 0.50%. In contrast, methanolic extracts of KNO_3-primed plants at 0.75% and GA_3-primed plants at 0.25% exhibited lower scavenging activities of 60 ± 1% and 60 ± 0.2%, respectively.

Among unprimed *O. basilicum* extracts, the highest radical-scavenging activity was observed in the petroleum ether extract (68 ± 0.1%), whereas the chloroform extract exhibited the lowest activity (25 ± 0.2%).

These findings indicate that antioxidant responses depended on both the priming treatment and extraction solvent, with three-day NTW treatment producing the highest activity among the reported treatment combinations.Phenolic compounds can contribute to antioxidant activity, and relationships between phenolic content and antioxidant measurements have been reported in herbs (Javanmardi *et al*., 2003; Zheng & Wang, 2001).

However, a correlation between TPC and DPPH activity was not established in the present study. Other extract constituents may also contribute to radical scavenging, and the two assays should be interpreted as complementary measurements.

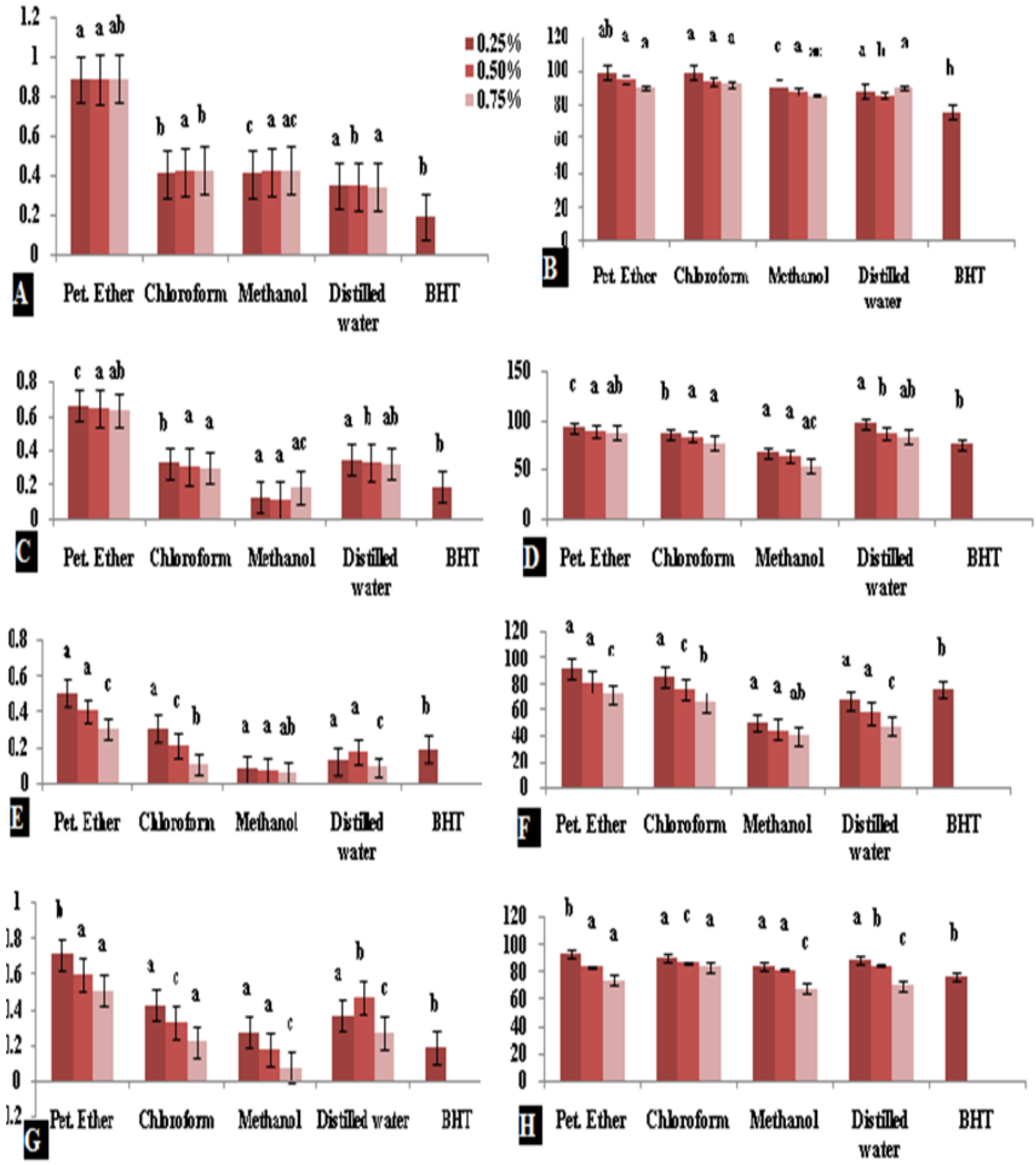

Fig 4a: Antioxidant effect under three different conc. of osmo-primed *O. basilicum* seed extracts, through DPPH assay at 517nm (A) Calcium chloride + absorption values (B) Calcium chloride + scavenging % values (C) Potassium chloride + absorption values (D) Potassium chloride + scavenging % values (E) Sorbitol + absorption values (F) Sorbitol + scavenging % values (G) Potassium nitrate + absorption values (H) Potassium nitrate + scavenging % values

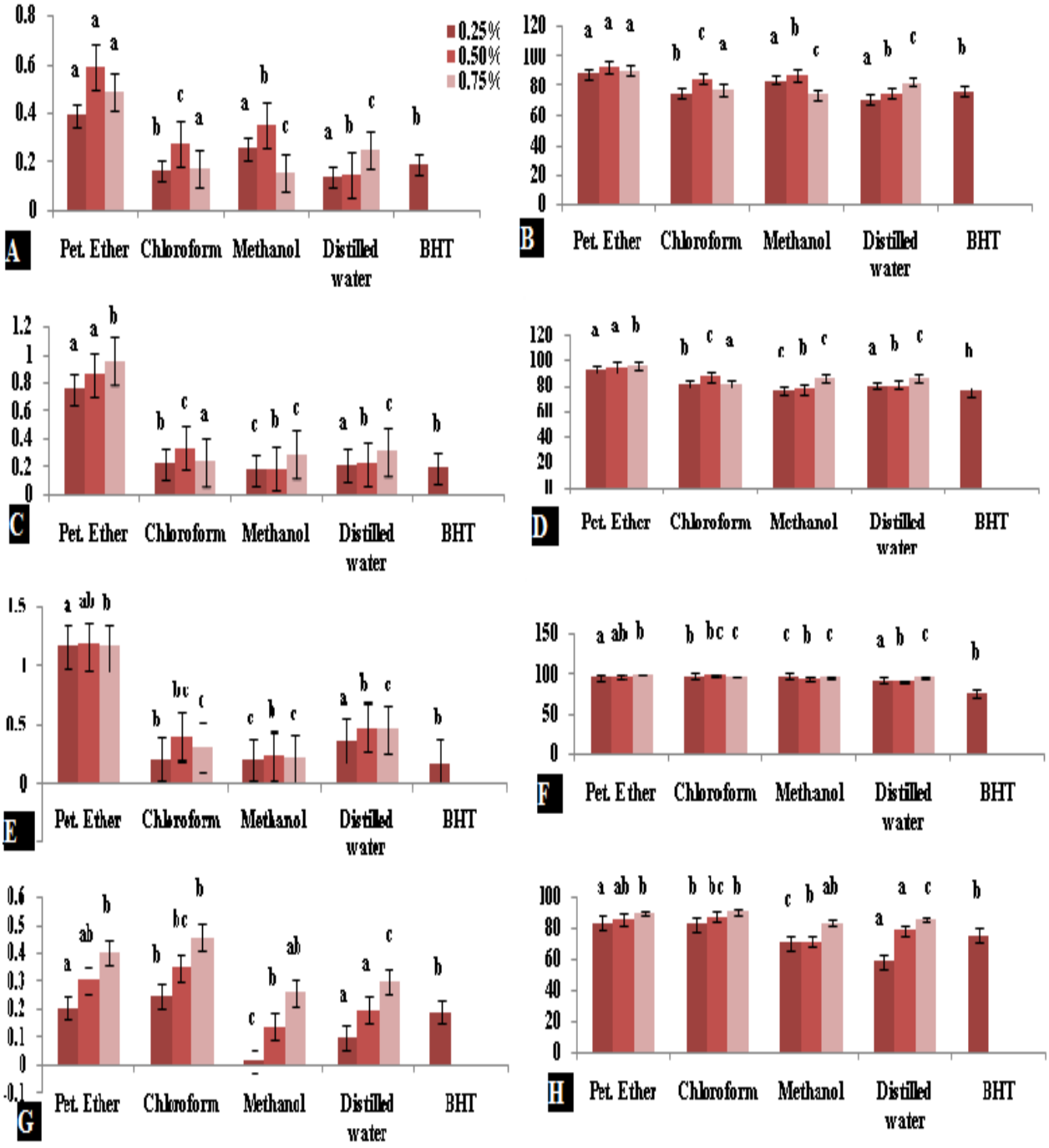

Fig 4b: Antioxidant effect under three different conc. of hormonal-primed *O. basilicum* seed extracts, through DPPH assay at 517nm (A) Gibberellic acid + absorption values (B) Gibberellic acid + scavenging % values (C) Auxin + absorption values (D) Auxin + scavenging % values (E) Cytokinin + absorption values (F) Cytokinin + scavenging % values (G) Ethylene + absorption values (H) Ethylene + scavenging % value

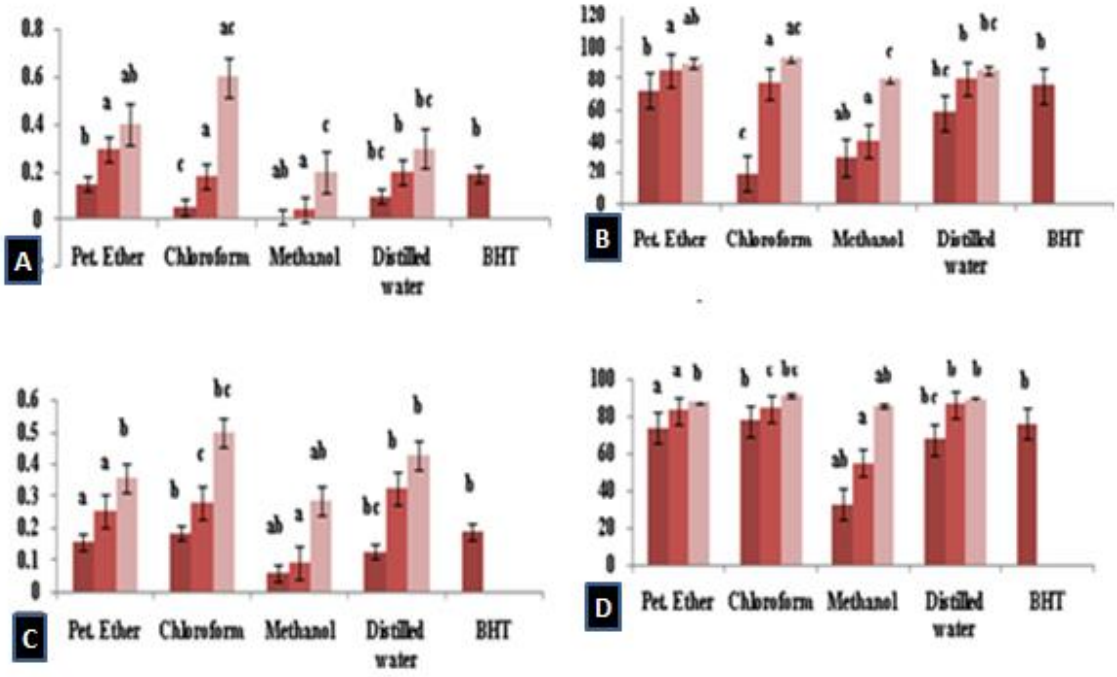

Fig 4c: Antioxidant effect under three different days of hydro and nano-primed *O. basilicum* seed extracts, through DPPH assay at 517nm (A) Hydro treated *O. basilicum* + absorption values (B) Hydro treated *O. basilicum* + scavenging % values (C) Nano treated *O. basilicum* + absorption values (D) Nano treated *O. basilicum* + scavenging % values

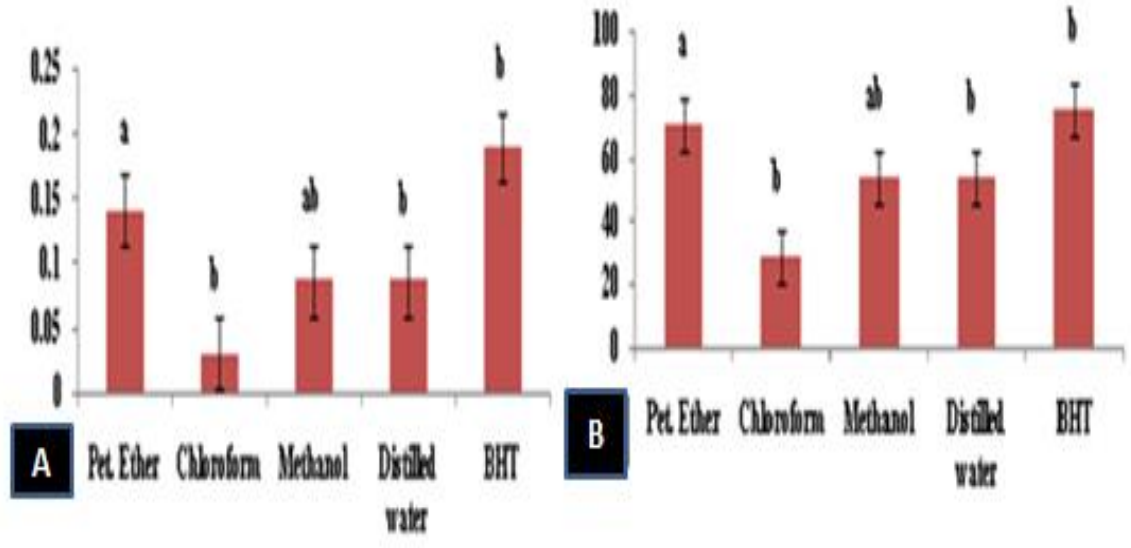

Fig 4d Antioxidant effect of *O. basilicum* seed under variable extracts, through DPPH assay at 517nm (A) *O. basilicum* + absorption values (B) *O. basilicum* + scavenging % values

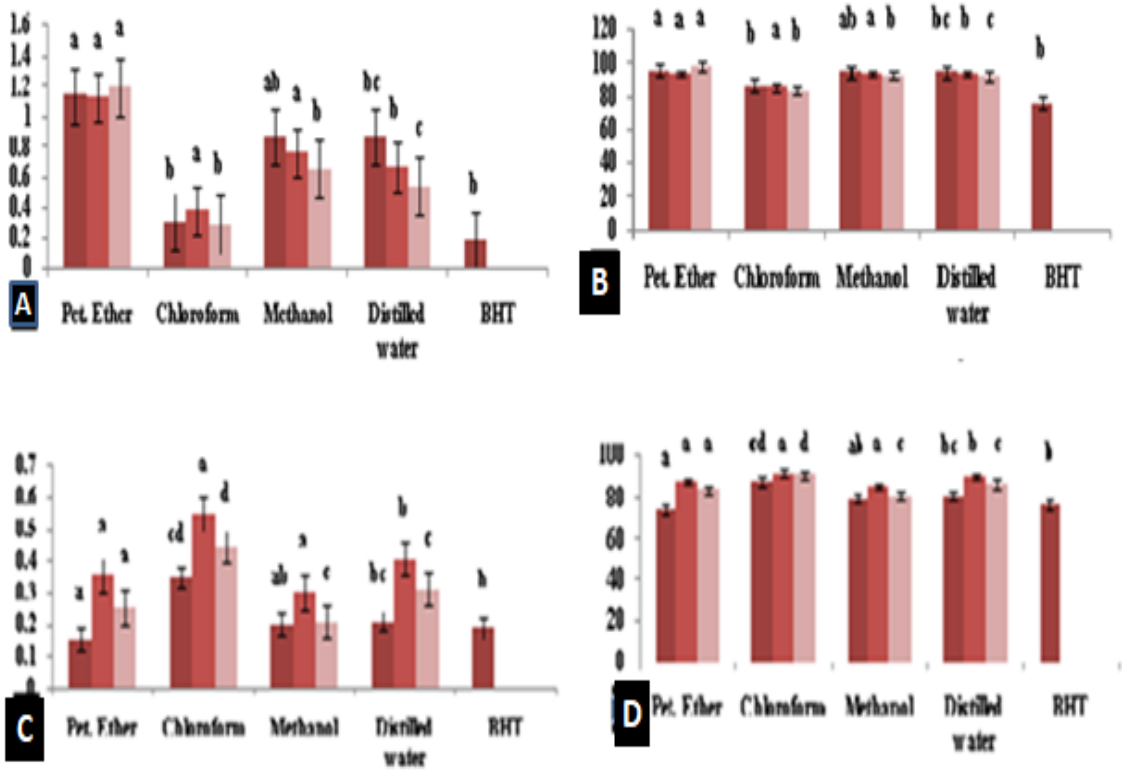

Fig 4e Antioxidant effect under three different conc. of chemically and halo-primed *O. basilicum* seed extracts, through DPPH assay at 517nm (A) H_2O_2 treated *O. basilicum* + absorption values (B) H_2O_2 treated *O. basilicum* + scavenging % values (C) NaCl treated *O. basilicum* + absorption values (D) NaCl treated *O. basilicum* + scavenging % values

3.8: Total Phenolic Content Assay among seed priming extracts of *O. basilicum*

Total phenolic content (TPC) of *O. basilicum* extracts varied among priming treatments and extraction solvents. Gallic acid was used to construct the calibration curve, and results were expressed as mg GAE/g dry extract. The highest TPC was reported for the methanolic extract following H_2O_2 priming at 0.75%. Methanolic extracts following three-day NTW treatment and three-day hydropriming exhibited TPC values of 95 ± 2^c and 90 ± 3^c mg GAE/g dry extract, respectively. Lower values were recorded for aqueous extracts of $CaCl_2$- and KNO_3-primed plants at 0.75%, with values of 43 ± 0.2^a and 45 ± 0.1^d mg GAE/g dry extract, respectively. These findings indicate that phenolic content differed among treatment–solvent combinations.

CONCLUSION

Seed priming influenced germination, morphological traits, and extracts bioactivity in *O. basilicum*, with responses depending on the treatment and measured outcome. H_2O_2 at 0.75% produced the highest reported speed of germination, while selected osmotic treatments and three-day NTW treatment also produced favorable germination responses. Selected H_2O_2, NTW, and hormonal treatments yielded favorable morphological, antimicrobial, and antioxidant responses. These findings identify promising priming approaches for further optimization, although their mechanisms and consistency across cultivation conditions require additional investigation.

DECLARATIONS

Author Contributions:

AY and MT conceptualized the review and drafted the manuscript; SK and ZS. Supervised, and reviewed the manuscript; NR, MR and ZM critically reviewed the manuscript and helped with the structure

Funding: The author states that no external funding was received for this research.

Conflicts of Interest: The authors declare no conflicts of interest

Declaration on the Use of Artificial Intelligence (AI):

The authors declare that any artificial intelligence (AI)-assisted technologies used during the preparation of this manuscript were employed solely to improve language, grammar, readability, or formatting. All scientific content, including the study design, data collection, analysis, interpretation of results, and conclusions, was conceived, verified, and approved by the authors. The authors accept full responsibility and accountability for the accuracy, originality, and integrity of the manuscript.

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