

# Enhancement of seed quality and bioactive compound accumulation in sunflower sprouts by dielectric barrier discharge plasma treatment

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**ABSTRACT:** Dielectric barrier discharge (DBD) plasma treatment has been reported to increase the quality of seeds and seedlings. In order to improve sprout quality, this study was conducted to investigate the effect of DBD plasma treatment on seed germination, vigour, growth, and bioactive compound content of sunflower (*Helianthus annuus*) sprouts. Two sunflower cultivars, Arfael and Jumbo, were treated using atmospheric DBD plasma at a discharge voltage of 20 kV for 15, 30, 60, 120, and 240 s. The non-treated seeds were used as control. The responses of seeds to the treatment depended on the exposure time and the cultivar. While the treatment stimulated seed germination and improved the vigour of the Jumbo cultivar at all exposure times, the Arfael cultivar responded only to exposures of 60 s, 120 s, and 240 s. Sprouts derived from 60 s, 120 s, and 240 s DBD plasma treatments exhibited greater dry weight than those from other treatments. Moreover, the Arfael sprouts showed a higher bioactive compound level than the Jumbo sprouts. The DBD plasma treatments of both cultivars for 120 s and 240 s enhanced the accumulation of total phenolics and flavonoids. However, plasma treatment did not affect DPPH radical scavenging. The results from this study indicate that the 120 s DBD plasma treatment is effective in enhancing seed germination, growth, and bioactive compound of sunflower sprouts. This study is the first of its kind to illustrate the potential benefits of the DBD plasma treatment to enhance the quality of sunflower sprouts.

**KEYWORDS:** DBD plasma, flavonoid, germination, phenolic, sunflower sprout

## INTRODUCTION

Dielectric barrier discharge (DBD) plasma is a non-thermal or cold plasma usually generated at low temperature and atmospheric pressure [1, 2]. It induces changes in plant chemistry while causing only minor damage to biological materials [1, 3–5]. Plasma treatment has been documented to improve seed performance and seedling growth in many plant species and is an attractive physical method, being chemical-free, environmentally friendly, quick, and non-destructive [2, 6]. The field and laboratory germination of *Lupinus angustifolius*, *Galega virginiana*, and *Melilotus albus* seeds subjected to plasma for 10 to 15 min increased by 10–20% compared with control [7]. The *Mimosa caesalpiniaefolia* seeds subjected to DBD plasma at

17.5 kV for 3 min exhibited a germination percentage eight times that of the untreated seeds [8]. The *Andrographis paniculata* seeds treated to DBD plasma at 4250 V for 10 s and 5950 V for 20 s had significantly improved germination index [6]. Exposing *Glycine max* seeds to plasma at 80 W for 15 s enhanced the germination index by 14.66% and the vigour index by 63.33% over control [9]. The shoot length of the *Raphanus sativus* seedlings after DBD plasma treatment at 9.2 kV for 180 s was 250% greater than control [10]. The sprouts of the 15-min non-thermal-plasma treated *Triticum aestivum* seeds showed higher growth than control [11]. DBD plasma also induces an increase in the concentration of bioactive compounds and is particularly effective at the seedling stage. The *Spinacia oleracea* seeds exposed to DBD plasma at

6 kV for 30 to 60 s produced seedlings with high concentrations of chlorophyll and phenolics [12]. Significant increases in phenolic content have also been reported for *T. aestivum* and *Capsicum annuum* seedlings grown from seeds DBD-plasma-treated for 60 s and 120 s, respectively [13, 14]. The plasma response of plant species, it appears, depends on factors including the discharge voltage and duration of exposure.

An unhealthy diet is a behavioural risk factor associated with non-communicable diseases (NCDs), including cardiovascular conditions, cancers, chronic respiratory diseases, and diabetes. Vegetable sprouts, a good source of vitamins, minerals, antioxidants, and dietary fibre, are considered to be one of the functional foods [15, 16]. Sprout cultivation requires no chemical treatment; it is simple and inexpensive. Being safe and healthy, sunflower (*Helianthus annuus*) sprouts are a popular green. However, the use of plasma treatment to promote the germination and accumulation of bioactive compounds in sunflower sprouts has not yet been reported. This study aimed to investigate the role of DBD plasma at different exposure times in sunflower seed germination and vigour. The growth, the total phenolic and flavonoid contents, and the DPPH radical scavenging of sunflower sprouts were recorded. DBD plasma treatment is a potentially useful alternative method for improving the bioactivity of this healthy food.

## MATERIALS AND METHODS

### DBD plasma treatment

DBD plasma was generated at atmospheric pressure between two parallel copper electrodes. Two glass plates were used as dielectric barriers. A gap between the two glass plates was set to 4 mm. The discharge voltage and frequency supplied to the electrodes were fixed at 20 kV and 5.5 kHz, respectively. The sunflower seeds were placed on the glass and exposed to atmospheric DBD plasma for 15, 30, 60, 120, and 240 s. The non-treated seeds were used as control.

### Seed preparation

The seeds of two sunflower cultivars, Arfael and Jumbo, were purchased from a wholesale market in the Pathum Thani Province, Thailand. They were sterilized using 1.5% (v/v) NaOCl for 4 min, then washed and soaked in tap water for 8 h. All the tests were conducted immediately after the preparation.

### Seed germination and vigour tests

The germination tests were conducted using four replications of 50 seeds. The seeds were germinated between rolled paper towels and placed in a germinator at 25 °C under fluorescent lighting for 8 h. The normal seedlings were evaluated at 4 and 10 days after sowing [17]. The germination percentage and germination percentage at first count (first count) were calculated. For mean time to germination (MTG), the seeds were germinated under the same test conditions. The normal seedlings were counted daily until 10 days after sowing. The MTG was calculated as  $MTG = \sum T_i N_i / \sum N_i$ , where  $N_i$  is the number of germinated seeds at time  $T_i$  [18].

### Sprout growth

One hundred seeds were seeded in 10 × 5 × 5 cm plastic boxes containing 3 cm in depth of moist peat moss. They were placed in a germinator at 25 °C under fluorescent lighting for 8 h. The sprouts were harvested 5 d after sowing, and the sprout length and fresh weight were recorded. To determine the dry weight, the sprouts were dried in a hot air oven at 50 °C for 72 h.

### Bioactive compound determination

For extraction, the dried sprouts were powdered and macerated in 95% ethanol, following the procedure of Worawattananutai et al [19]. The ratio of dry sample to ethanol was 1:10 weight by volume. The powder was macerated for 72 h and passed through filter paper. The residues were further macerated twice. The combined extracts were evaporated in the hot air oven at 50 °C for 72 h. The total phenolic and flavonoid contents of the extracts were measured, and the antioxidant activity was determined using the DPPH radical scavenging assay.

### Total phenolic content

The total phenolic content was determined using Folin-Ciocalteu colorimetric method as described by Jirakiattikul et al [20]. The extract was dissolved in absolute ethanol to a concentration of 1 mg/ml and sonicated for 1 min. Twenty µl of each sample was mixed with 100 µl of 10 times diluted Folin Ciocalteu's reagent, then 80 µl of 7.5% Na<sub>2</sub>CO<sub>3</sub> was added and the mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured in a microplate reader (Power Wave XS, Biotek) at 765 nm. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry extract.

### Total flavonoid content

The total flavonoid content was assessed using a modified method of Zhu et al [21]. The extract was dissolved in absolute ethanol to a concentration of 1 mg/ml and sonicated for 1 min. Five hundred  $\mu$ l of each sample was mixed with 75  $\mu$ l of 5% NaNO<sub>2</sub> and left for 6 min, then 150  $\mu$ l of 10% AlCl<sub>3</sub> was added and the mixture was left for 6 min. Finally, 500  $\mu$ l of 1 M NaOH and 275  $\mu$ l of distilled water were added, and the mixture was incubated at room temperature for 15 min. Absorbance was measured in a microplate reader at 510 nm. The total flavonoid content was expressed as mg catechin equivalent (CE)/g dry extract.

### Antioxidant activity

The antioxidant activity was measured using the DPPH radical scavenging assay adapted from Yamasaki et al [22]. The DPPH solution was prepared by dissolving 12 mg DPPH with 50 ml ethanol. The extract was dissolved in absolute ethanol to a concentration of 1 mg/ml, then 100  $\mu$ l of each sample was mixed with 100  $\mu$ l of DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was measured in a microplate reader at 520 nm. The percentage inhibition was calculated as % inhibition =  $[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$ . Here,  $Abs_{control}$  is the absorbance of the control, and  $Abs_{sample}$  is the absorbance of the test sample.

### Statistical analysis

The experiments used a 2  $\times$  6 factorial CRD with four replications. The data were analysed using the SPSS software. The mean separation was conducted by applying Duncan's multiple range test at  $p \leq 0.05$ .

## RESULTS

The interaction effect of the cultivar and DBD plasma exposure time showed significant differences across all parameters, except for sprout dry weight and DPPH radical scavenging (Tables 1–3).

### Seed germination and vigour

The Jumbo seeds exposed to the DBD plasma treatment exhibited germination percentages of 7.27% to 10.30% higher than that of control. In contrast, no significant difference with control was found for the Arfael seeds subjected to DBD plasma treatment at any exposure time. First count and MTG were used as indicators of seed vigour. First count of Jumbo seeds treated using DBD plasma at all the

different exposure times exceeded that of control by 10.94% to 15.32%. The Arfael seeds treated for 60 s, 120 s, and 240 s were respectively 4.86%, 6.94%, and 7.64% above control. The Arfael seeds exposed for 120 s showed the lowest MTG, though this was not statistically different from the 15 s, 30 s, 60 s, or 240 s exposures, or the Jumbo seeds exposed for 240 s. The longer MTG was observed in the Jumbo seeds exposed for 15 s to 120 s and the non-treated seeds of both cultivars (Table 1).

### Sprout growth

The DBD plasma exposure for 60 s, 120 s, and 240 s increased the sprout length of the Arfael seedlings by 51.94%, 46.53%, and 55.49%, respectively, compared with control. The Jumbo sprouts were generally short, and only the 30 s, 60 s, and 120 s DBD plasma treatments produced sprout lengths greater than control. The sprout fresh weight of the Jumbo seedlings showed significant increases of 15.91%, 22.48%, and 13.95% over control when exposed to the DBD plasma for 30 s, 60 s, and 120 s, respectively. Following the DBD plasma treatment, the sprout fresh weight of 'Arfael' seedlings was below that of the control, with the exception of the 120 s treatment. The sunflower sprouts developed from the 60 s, 120 s, and 240 s DBD plasma-treated seeds exhibited higher growth, as the sprout dry weight increased over control of 10.61%, 17.72%, and 11.72%, respectively. The sprout dry weights of the Jumbo seedlings were greater than those of the Arfael (Table 2).

### Bioactive compounds

Compared with control, the DBD plasma exposure of 120 s and 240 s respectively enhanced the total phenolic content by 19.71% and 20.80% in Jumbo and 7.07% and 7.96% in Arfael. The optimal exposure times for improving the total flavonoid content differed between the cultivars. Compared with control, the Jumbo sprouts grown from the seeds treated for 15 s, 60 s, and 240 s increased by 33.24%, 23.73%, and 19.90%, respectively. The Arfael sprouts grown from seeds treated for 30 s by 14.90%, and for 120 s by 18.15%. The inhibition percentage of DPPH was not statistically different among the DBD plasma treatments. Overall, Arfael showed stronger antioxidant activity than Jumbo (Table 3).

## DISCUSSION

Seed germination and vigour are important for sprout cultivation. Pre-sowing treatment is usually

**Table 1** Germination percentage, first count, and mean time to germination (MTG) of the Jumbo and Arfael sunflower seeds subjected to DBD plasma treatments at different exposure times.

Cultivar	DBD plasma exposure time (s)	Germination (%)	Seed vigour	
			First count (%)	MTG (days)
Jumbo		88.75 ± 3.48 <sup>a</sup>	75.83 ± 4.17	3.58 ± 0.11 <sup>a</sup>
Arfael		82.58 ± 1.82 <sup>b</sup>	74.75 ± 2.63	3.45 ± 0.09 <sup>b</sup>
	Control	82.25 ± 2.25 <sup>b</sup>	70.25 ± 3.11 <sup>b</sup>	3.60 ± 0.08 <sup>a</sup>
	15	85.50 ± 3.66 <sup>a</sup>	75.75 ± 2.49 <sup>a</sup>	3.52 ± 0.14 <sup>ab</sup>
	30	87.00 ± 4.28 <sup>a</sup>	75.00 ± 3.55 <sup>a</sup>	3.49 ± 0.10 <sup>bc</sup>
	60	86.50 ± 4.63 <sup>a</sup>	77.25 ± 2.82 <sup>a</sup>	3.53 ± 0.15 <sup>ab</sup>
	120	86.50 ± 4.99 <sup>a</sup>	76.50 ± 2.07 <sup>a</sup>	3.51 ± 0.13 <sup>abc</sup>
	240	86.25 ± 3.92 <sup>a</sup>	77.00 ± 1.51 <sup>a</sup>	3.43 ± 0.05 <sup>c</sup>
Jumbo	Control	82.50 ± 2.52 <sup>b</sup>	68.50 ± 3.42 <sup>d</sup>	3.61 ± 0.08 <sup>a</sup>
	15	88.50 ± 1.91 <sup>a</sup>	77.00 ± 2.58 <sup>ab</sup>	3.62 ± 0.10 <sup>a</sup>
	30	90.50 ± 1.91 <sup>a</sup>	78.00 ± 1.63 <sup>ab</sup>	3.55 ± 0.07 <sup>ab</sup>
	60	90.50 ± 1.91 <sup>a</sup>	79.00 ± 2.58 <sup>a</sup>	3.63 ± 0.12 <sup>a</sup>
	120	91.00 ± 1.15 <sup>a</sup>	76.00 ± 2.83 <sup>ab</sup>	3.61 ± 0.09 <sup>a</sup>
	240	89.50 ± 2.52 <sup>a</sup>	76.50 ± 1.91 <sup>ab</sup>	3.44 ± 0.08 <sup>bc</sup>
Arfael	Control	82.00 ± 2.31 <sup>b</sup>	72.00 ± 1.63 <sup>c</sup>	3.59 ± 0.09 <sup>a</sup>
	15	82.50 ± 1.91 <sup>b</sup>	74.50 ± 1.91 <sup>bc</sup>	3.42 ± 0.09 <sup>c</sup>
	30	83.50 ± 2.52 <sup>b</sup>	72.00 ± 1.63 <sup>c</sup>	3.42 ± 0.07 <sup>c</sup>
	60	82.50 ± 1.91 <sup>b</sup>	75.50 ± 1.91 <sup>ab</sup>	3.42 ± 0.08 <sup>c</sup>
	120	82.00 ± 1.63 <sup>b</sup>	77.00 ± 1.15 <sup>ab</sup>	3.41 ± 0.03 <sup>c</sup>
	240	83.00 ± 1.15 <sup>b</sup>	77.50 ± 1.00 <sup>ab</sup>	3.43 ± 0.02 <sup>bc</sup>
Cultivar (A)		**	ns	**
DBD plasma (B)		**	**	*
A × B		**	*	*

Means ± SD within each column followed by the same letters are not significantly different at  $p \leq 0.05$  level by DMRT; ns non-significantly different at  $p > 0.05$ ; \*\* significantly different at  $p \leq 0.01$ ; \* significantly different at  $p \leq 0.05$ .

required to increase sprout uniformity. Since it is quick, non-destructive, environmentally friendly, and requires no use of chemicals, cold plasma treatment is a widely-used method [2, 6]. The response of the sunflower seeds to the DBD plasma treatment depended on the cultivar and the exposure time. The plasma treatment stimulated seed germination and increased the vigour of the Jumbo sprouts at all exposure times. Similar results have been reported for *G. max* seeds exposed to plasma at 80 W for 15 s, enhancing seed germination by 14.66% and the vigour index by 63.33% compared with control [9]. The plasma treatment of *Carthamus tinctorium* increased the germination percentage by 50% over control while reducing germination time by 24 h [23]. In contrast, no significant difference was found in the germination percentage of the plasma treated Arfael seeds compared with control. However, the DBD plasma treatment for 60 s, 120 s, and 240 s increased the seed vigour. These

results were in agreement with those reported for *T. aestivum* [11] and *A. paniculata* [6]. In both cases, plasma treatment improved only seed vigour. The effect of DBD plasma on seed germination and vigour is attributed to the modification of the physical structure of the seed coat. Šerá et al [24] demonstrated that longitudinal cracks appeared in the caryopsis surface of *T. aestivum* following plasma treatment. When the *C. tinctorium* seeds were plasma-treated, tiny holes occurred on the surface of the seed coat and a hilum with a soft structure developed [23]. The seed coat also became hydrophilic, improving wettability [8, 11] and increasing water imbibition and oxygen penetration [6, 9, 12, 23]. Initial seed germination is usually characterized by a rapid uptake of water. Increased water absorption and gas exchange through the cracked seed coat enhances germination in the plasma-treated seeds.

Exposure to DBD plasma for 60 s, 120 s, and 240 s resulted in the improvement of sunflower

**Table 2** Length, fresh weight, and dry weight of the Jumbo and Arfael sunflower sprouts derived from DBD plasma-treated seeds at different exposure times.

Cultivar	DBD plasma exposure time (s)	Length (cm)	Fresh weight (mg/plant)	Dry weight (mg/plant)
Jumbo		6.52 ± 0.87 <sup>b</sup>	225.08 ± 34.54	37.70 ± 4.55 <sup>a</sup>
Arfael		7.87 ± 1.29 <sup>a</sup>	216.11 ± 27.26	25.45 ± 3.96 <sup>b</sup>
	Control	6.08 ± 0.66 <sup>d</sup>	223.46 ± 30.95 <sup>ab</sup>	29.86 ± 9.26 <sup>bc</sup>
	15	6.60 ± 0.61 <sup>cd</sup>	199.97 ± 23.61 <sup>bc</sup>	28.61 ± 7.15 <sup>c</sup>
	30	7.05 ± 0.49 <sup>cb</sup>	222.52 ± 26.86 <sup>ab</sup>	29.44 ± 5.07 <sup>bc</sup>
	60	8.47 ± 0.63 <sup>a</sup>	241.46 ± 29.74 <sup>a</sup>	33.03 ± 6.57 <sup>ab</sup>
	120	7.59 ± 1.33 <sup>b</sup>	240.62 ± 20.22 <sup>a</sup>	35.15 ± 6.65 <sup>a</sup>
	240	7.37 ± 1.99 <sup>b</sup>	195.56 ± 28.23 <sup>c</sup>	33.36 ± 9.34 <sup>ab</sup>
Jumbo	Control	6.26 ± 0.77 <sup>fg</sup>	211.09 ± 39.71 <sup>bcd</sup>	38.41 ± 0.39
	15	6.10 ± 0.34 <sup>fg</sup>	215.99 ± 14.4 <sup>bcd</sup>	35.01 ± 1.89
	30	6.70 ± 0.19 <sup>def</sup>	244.68 ± 11.94 <sup>ab</sup>	34.05 ± 1.23
	60	7.97 ± 0.22 <sup>bc</sup>	258.56 ± 15.00 <sup>a</sup>	38.15 ± 2.39
	120	6.52 ± 0.62 <sup>ef</sup>	240.55 ± 27.57 <sup>ab</sup>	40.27 ± 5.31
	240	5.56 ± 0.47 <sup>g</sup>	179.63 ± 27.96 <sup>d</sup>	40.29 ± 8.58
Arfael	Control	5.91 ± 0.58 <sup>fg</sup>	235.83 ± 15.83 <sup>abc</sup>	21.31 ± 2.27
	15	7.11 ± 0.27 <sup>de</sup>	183.94 ± 20.17 <sup>d</sup>	22.22 ± 2.54
	30	7.40 ± 0.43 <sup>cd</sup>	200.35 ± 15.18 <sup>cd</sup>	24.83 ± 1.39
	60	8.98 ± 0.46 <sup>a</sup>	224.37 ± 32.54 <sup>abc</sup>	27.90 ± 5.01
	120	8.66 ± 0.84 <sup>ab</sup>	240.69 ± 13.91 <sup>ab</sup>	30.02 ± 2.19
	240	9.19 ± 0.50 <sup>a</sup>	211.50 ± 20.03 <sup>bcd</sup>	26.43 ± 1.43
Cultivar (A)		**	ns	**
DBD plasma (B)		**	*	*
A × B		**	*	ns

Means ± SD within each column followed by the same letters are not significantly different at  $p \leq 0.05$  level by DMRT; ns non-significantly different at  $p > 0.05$ ; \*\* significantly different at  $p \leq 0.01$ ; \* significantly different at  $p \leq 0.05$ .

sprout growth. These exposure times may stimulate the biochemical pathways during germination by triggering release of hormones including GA<sub>3</sub> (gibberellin) and a range of hydrolytic enzymes [12]. The supporting nutrients stored in the seeds may then be utilized more effectively, hence enhancing plant growth [9, 12]. The stimulation of plant growth by plasma treatment of seeds has been documented both in the seedling growth phase [9–11, 14, 25–27] and in the mature growth phase in the field [25, 28]. Conversely, plasma treatment has been reported to reduce seedling growth in some plant species, including *Fagopyrum esculentum* [29], *T. aestivum*, and *Avena sativa* [24].

The total phenolic and flavonoid enhancement of sunflower sprouts depended on the cultivars and the exposure time. Arfael sprouts had a higher level of bioactive compounds than Jumbo sprouts. Longer DBD plasma exposure times of 120 s and 240 s produced stronger responses. Plasma gen-

erates reactive oxygen species (ROS), heat, nitric oxide, and UV radiation [13, 27]. Šerá et al [24] proposed that ROS penetrates the seed coat and affects the metabolic processes of the cells. For self-protection, each cell increases the accumulation of bioactive compounds. Our results were in agreement with the previous reports. Following the DBD plasma treatment of *S. oleracea* [12] and *T. aestivum* seeds [13] for 30–60 s and 60 s, respectively, the seedlings exhibited significantly increased phenolic content. Iranbakhsh et al [14] demonstrated an 82.3% increase over control in the phenolic content of *C. annuum* seedling leaves grown from 120 s DBD plasma-treated seeds. Moreover, the sprouts derived from the DBD plasma-treated seeds are safe without microbial contaminations. Several reports have been documented in this issue; for instance, Mitra et al [30] exposed *Cicer arietinum* seeds to cold atmospheric plasma. Their results showed a significant reduction of natural surface microorgan-

**Table 3** Total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging capacity of the Jumbo and Arfael sunflower sprouts derived from DBD plasma-treated seeds at different exposure times.

Cultivar	DBD plasma exposure time (s)	TPC (mg GAE/g dry extract)	TFC (mg CE/g dry extract)	DPPH radical scavenging capacity (%)
Jumbo		60.35 ± 6.17 <sup>b</sup>	30.25 ± 4.08 <sup>b</sup>	90.78 ± 3.23 <sup>b</sup>
Arfael		91.83 ± 3.56 <sup>a</sup>	44.96 ± 4.65 <sup>a</sup>	93.42 ± 1.55 <sup>a</sup>
	Control	72.68 ± 17.05 <sup>c</sup>	33.69 ± 9.05 <sup>b</sup>	91.64 ± 3.64
	15	72.37 ± 17.74 <sup>c</sup>	37.35 ± 4.31 <sup>a</sup>	92.92 ± 3.09
	30	75.84 ± 19.62 <sup>b</sup>	37.94 ± 10.45 <sup>a</sup>	92.21 ± 1.28
	60	72.69 ± 18.21 <sup>c</sup>	39.19 ± 7.85 <sup>a</sup>	90.66 ± 4.49
	120	81.42 ± 14.53 <sup>a</sup>	38.76 ± 11.30 <sup>a</sup>	92.01 ± 1.78
	240	82.12 ± 14.67 <sup>a</sup>	38.68 ± 8.61 <sup>a</sup>	93.15 ± 1.30
Jumbo	Control	56.78 ± 1.37 <sup>d</sup>	26.08 ± 4.21 <sup>f</sup>	90.39 ± 5.04
	15	55.82 ± 1.25 <sup>d</sup>	34.75 ± 2.81 <sup>d</sup>	90.77 ± 1.04
	30	57.16 ± 23.84 <sup>d</sup>	28.41 ± 1.20 <sup>ef</sup>	91.54 ± 0.50
	60	55.79 ± 2.10 <sup>d</sup>	32.27 ± 2.87 <sup>de</sup>	88.29 ± 5.63
	120	67.97 ± 2.97 <sup>c</sup>	28.71 ± 2.39 <sup>ef</sup>	90.53 ± 1.13
	240	68.59 ± 2.55 <sup>c</sup>	31.27 ± 4.66 <sup>def</sup>	93.17 ± 1.82
Arfael	Control	88.59 ± 1.54 <sup>b</sup>	41.31 ± 4.33 <sup>bc</sup>	92.89 ± 1.13
	15	88.91 ± 1.46 <sup>b</sup>	39.94 ± 4.18 <sup>c</sup>	95.07 ± 2.98
	30	93.40 ± 2.77 <sup>a</sup>	47.47 ± 3.33 <sup>a</sup>	92.88 ± 1.54
	60	89.58 ± 2.85 <sup>b</sup>	46.12 ± 2.75 <sup>ab</sup>	93.02 ± 0.59
	120	94.86 ± 1.43 <sup>a</sup>	48.81 ± 4.78 <sup>a</sup>	93.50 ± 0.42
	240	95.64 ± 2.74 <sup>a</sup>	46.08 ± 2.28 <sup>ab</sup>	93.14 ± 0.80
Cultivar (A)		**	**	**
DBD plasma (B)		**	*	ns
A × B		**	**	ns

Means ± SD within each column followed by the same letters are not significantly different at  $p \leq 0.05$  level by DMRT; ns non-significantly different at  $p > 0.05$ ; \*\* significantly different at  $p \leq 0.01$ ; \* significantly different at  $p \leq 0.05$ .

ism contamination under 2 min and 5 min treatments. Butscher et al [4] also studied the impact of non-thermal plasma treatment for 5 min and 10 min on artificially applied *Escherichia coli* on *Allium cepa*, *Medicago sativa*, *Lepidium sativum*, and *R. sativus* seeds. The inactivation efficiency of *E. coli*, they concluded, increased with treatment time. Moreover, Štěpánová et al [5] showed that atmospheric pressure plasma treatment was able to reduce some of the microorganisms and pathogens affecting the seeds of *Cucumis sativus* and *C. annuum*.

The results from this study indicate that the DBD plasma exposure time of 120 s is optimal in enhancing the quality of sunflower seeds and sprouts. A high percentage of germination, short germination time, and non-pathogen contamination are the important seed quality factors for sprout production. Consequently, pre-sowing seed treatment is normally applied to produce high sprout

quality. The seed treatment operated by physical technique requires specific equipment. In the case of DBD plasma equipment, it consists of the following components: a power supply system, US\$ 1000; electrode plates, US\$ 66.67; signal generator, US\$ 333.33; and high voltage coil, US\$ 100. Thus, the starting cost of the DBD plasma equipment is US\$ 1500. For enhancing sprout quality, the appropriate exposure time of DBD plasma is 120 s. Therefore, the operating cost of electric power ( $0.5 \text{ kW} \times 0.03 \text{ h} \times \text{US\$ } 0.167/\text{kW h}$ ) is US\$ 0.0025 per treatment (1 treatment = 100 seeds). Due to the advantage of the DBD plasma technique in terms of being chemical-free and environmentally friendly, the expense for the DBD plasma equipment and operating cost is low comparing with the common pre-sowing seed treatments, such as soaking seeds in water or a solution of inorganic substances. These seed treatments typically produce waste that causes environmental concerns [30].

## CONCLUSION

This study showed the potential benefits of DBD plasma treatment to improve sunflower seed performance and sprout quality. The optimal DBD plasma exposure time of 120 s could enhance the germination percentage, reduce germination time, improve sprout growth, and increase the total phenolic (including flavonoid) contents in sunflower sprouts. To produce high bioactivity sunflower sprouts for functional foods, it is recommended that the seeds should be treated with DBD plasma for 120 s before sprouting.

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