



Cold plasma jet to enhance fruiting body production and bioactive phytochemicals from mutant *Cordyceps militaris*

Presented by

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Outline

□ Background

- Project beginning
- White *C. militaris*

□ Experimental method

- Plasma jet device
- Plasma treatment on White *C. militaris*

□ Results and Discussions

- Plasma Characteristics
- Plasma treatment on solution

characteristics

□ Results and Discussions

- Plasma treatment on fruiting body production, cordycepin, and adenosine
- Antioxidant enzymes and lipid peroxidation
- β -glucan and bioactive compounds
- Antioxidant activities

□ Conclusion

Background: Project beginning

- Kasetbuddy farm is a local farm in Saraburi Province: Distributor of *Cordyceps militaris* (*C. militaris*).
- Dry weight cost is ~1,000-2,000\$/kg



Background: Project beginning

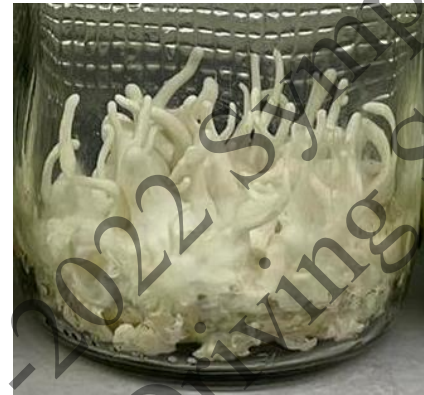
- ACX herb cordyceps farm (Prachin Buri Province): Distributor of *Cordyceps militaris* (*C. militaris*) with high cordycepin content (~2000 mg/100g).
- Mutant *C. militaris* is white *C. militaris*.



Background: *White C. militaris*

Mutant *C. militaris*

High contents of SOD GST and beta-glucan



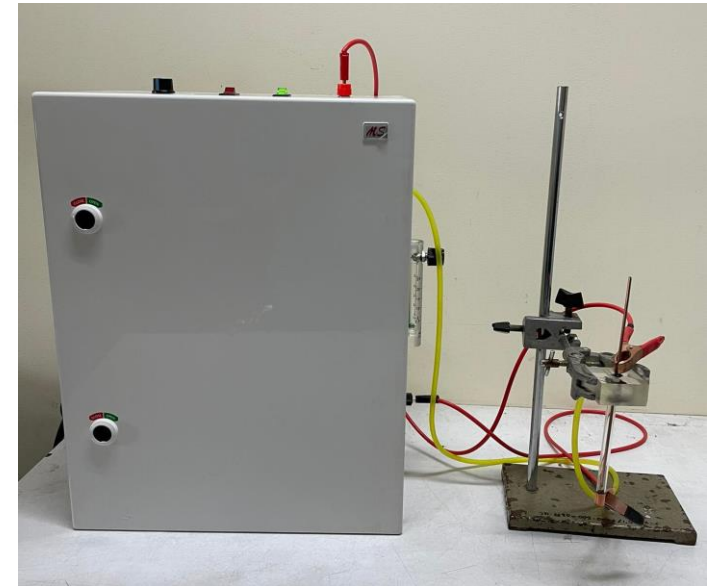
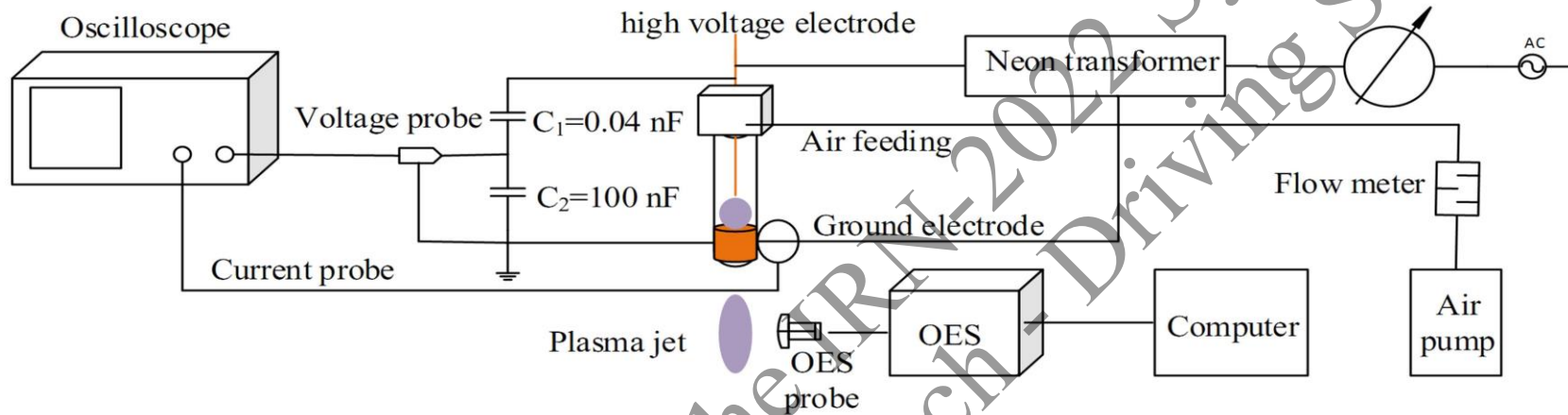
Low cordycepin content

Low yield

Low germination

Experimental Method: Plasma jet device

Schematic of the plasma apparatus



Experimental Method: Plasma treatment on White *C. militaris*

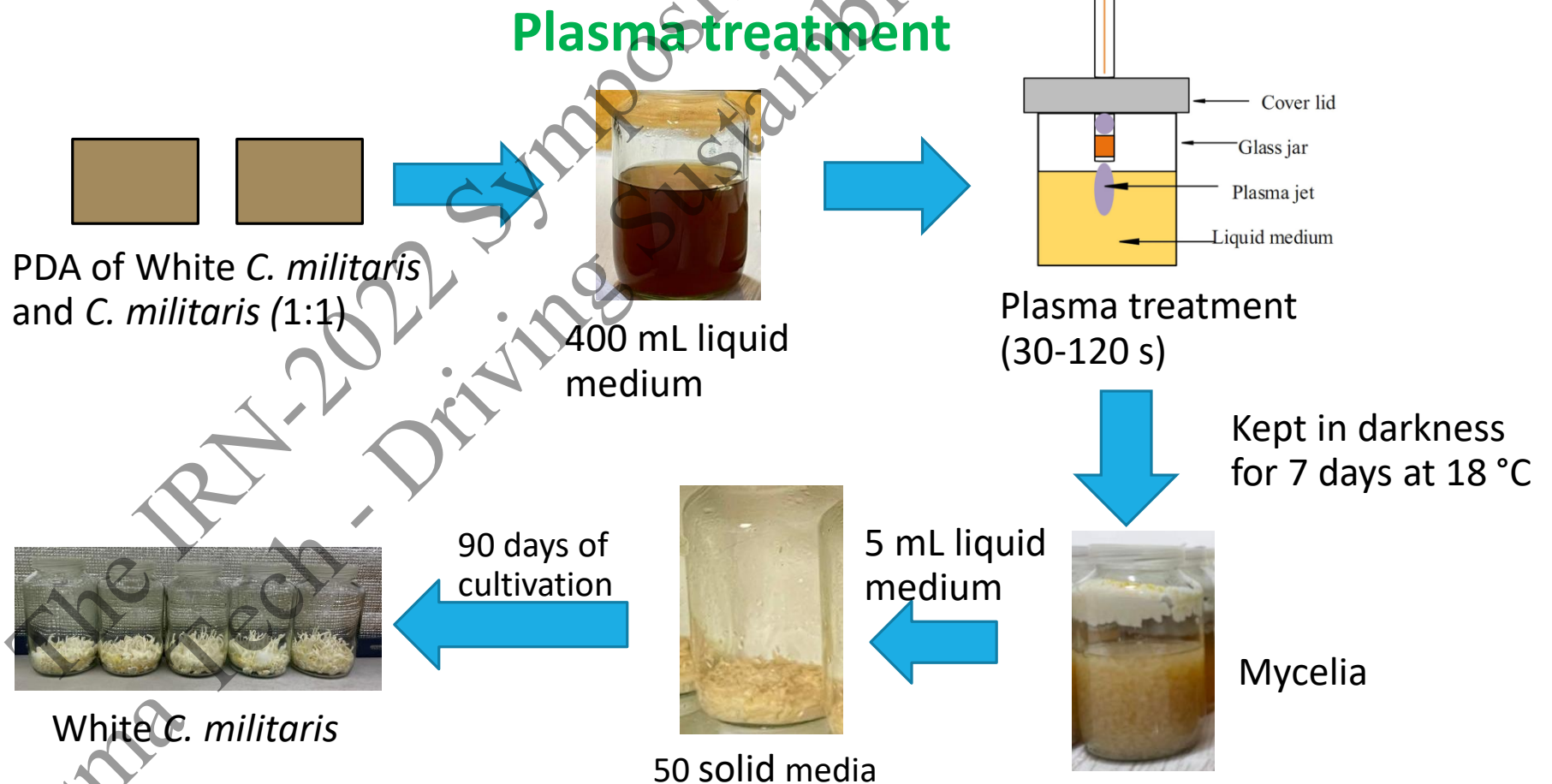
C. militaris cultivation



Liquid medium



Solid medium



Experimental Method: Plasma treatment on White *C. militaris*

Plasma exposure to liquid medium



A liquid medium transferred to 50 solid media

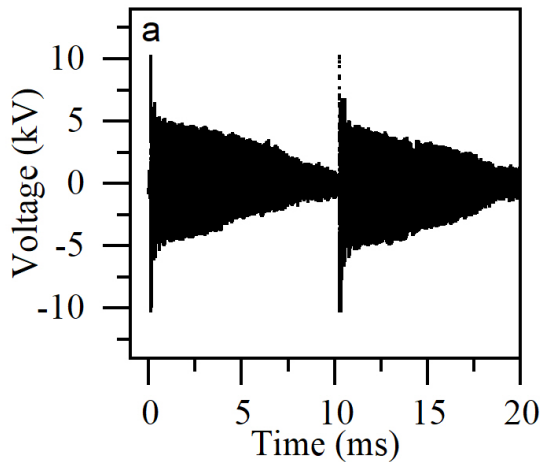


White *C. militaris* during cultivation

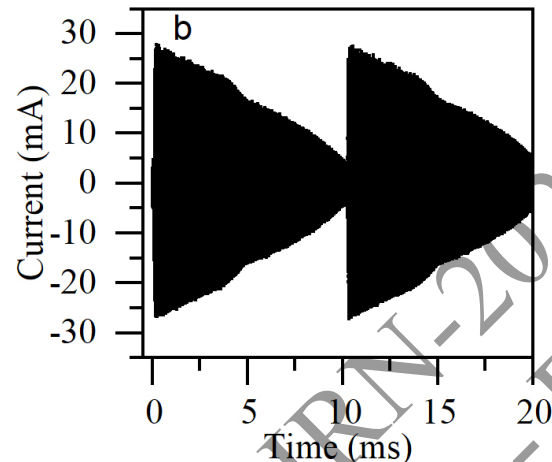


Results and Discussions: Plasma Characteristics

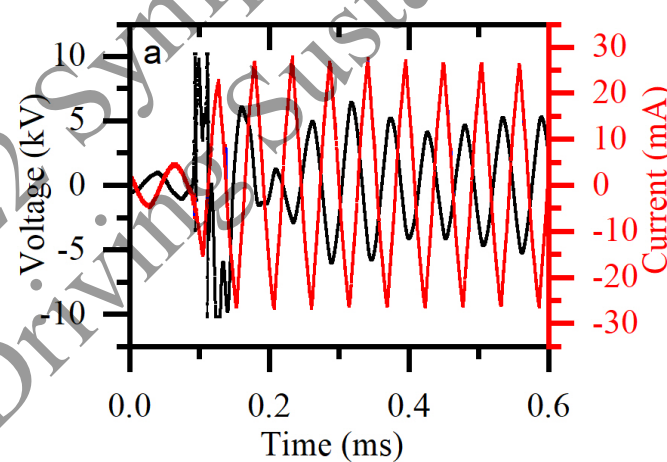
Characteristics of voltage and current under air Plasma at a fixed flow rate of 5 L/min.



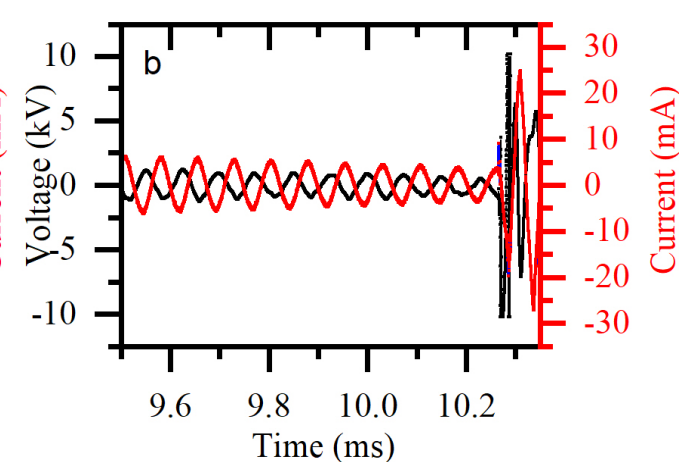
Pulse-modulation waveform



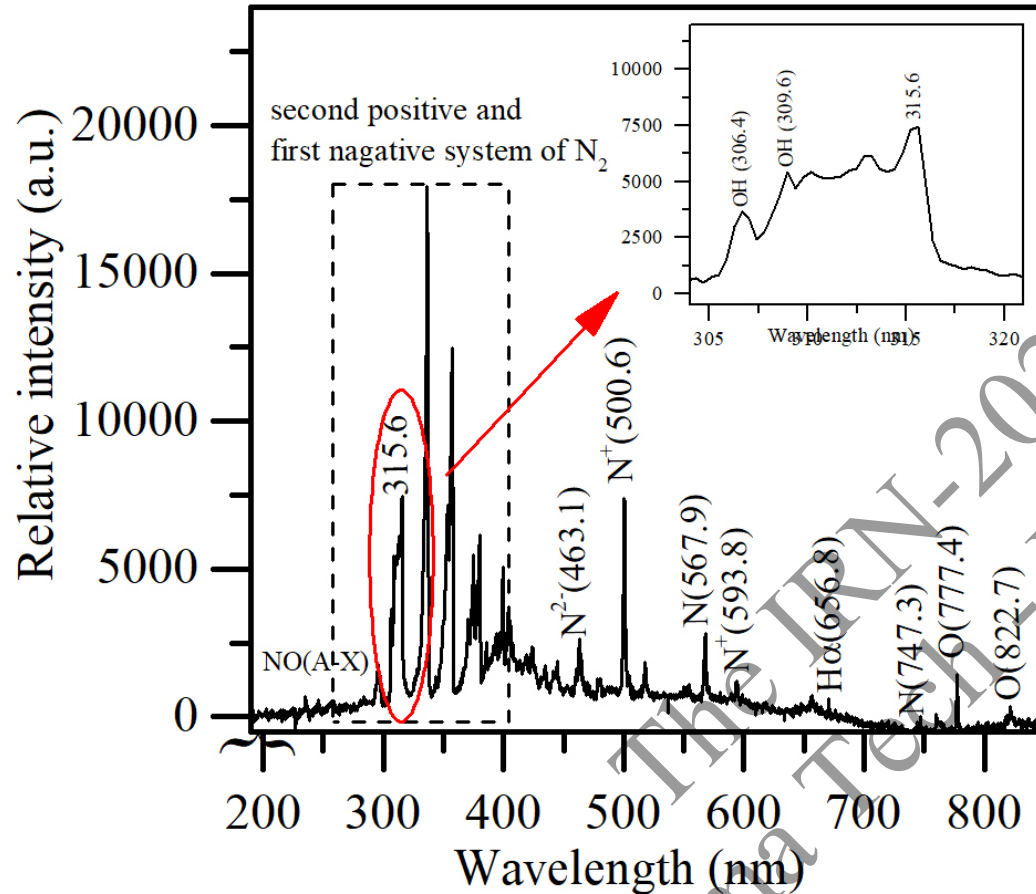
Ignition phase



Extinguishment phase



Results and Discussions: Plasma Characteristics



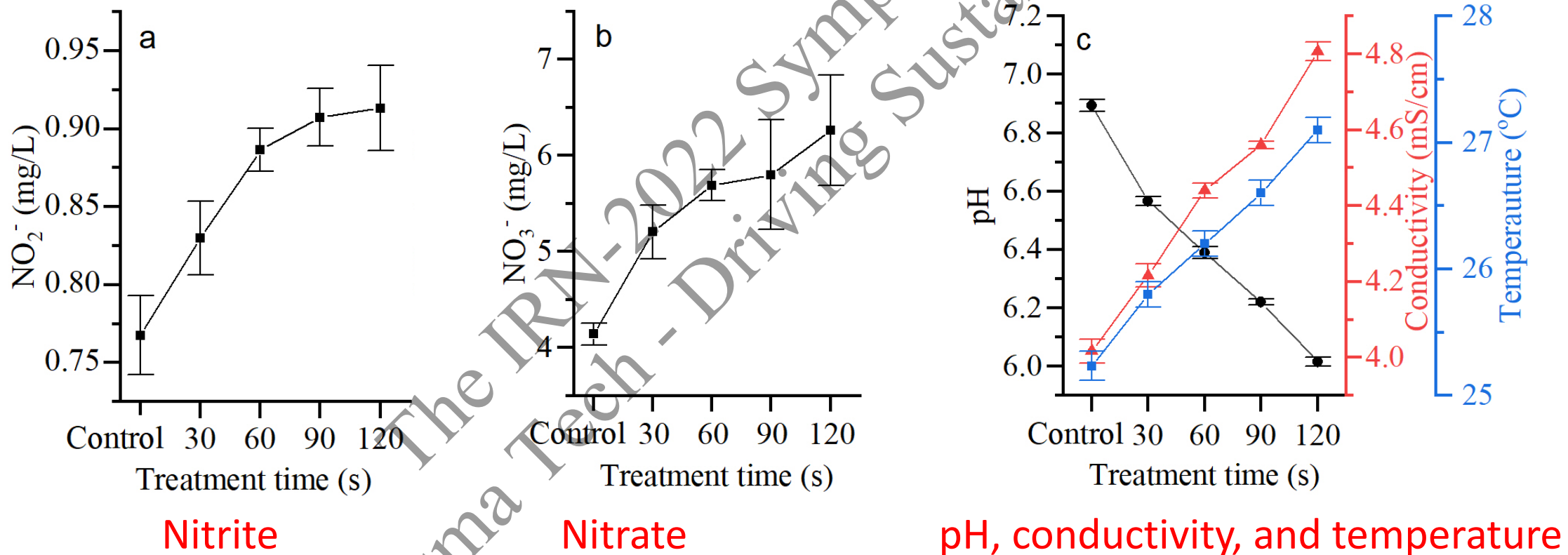
Optical emission spectra of the air CPJ

- N₂ second positive system, N₂ first negative system
- The nitrogen ion (N⁺)
- OH bands
- Nitric oxides (NO)
- Oxygen atoms

Seelarat et al., 2022. Plasma Chemistry and Plasma Processing. DOI :10.1007/s11090-022-10292-w.

Results and Discussions: Plasma treatment on solution characteristics

Effects of plasma jet treatment on solution characteristics



Results and Discussions: Plasma treatment on fruiting body production, cordycepin, and adenosine

Treatment time (s)	Fresh weight (g)	Dry weight (g)	Cordycepin (mg/100 g)	Adenosine (mg/100 g)
CK1	18.38±2.17a	3.29±0.39a	834.56±23.94a	72.53±2.37ab
CK2	21.30±4.72b	3.59±0.80ab	939.42±19.33b	77.59±1.94a
30	26.09±3.50c	4.40±0.59c	1176.46±56.47c	75.99±3.63a
60	21.46±5.47b	3.95±0.36b	1062.50±12.52d	72.92±4.10a
90	20.91±1.92b	3.84±0.98b	982.79±49.77bd	70.10±4.40b
120	21.69±2.57b	3.66±0.43ab	877.27±20.81ab	54.50±4.07c

CK1: Control of white *C. militaris*, CK2: Control of blended *C. militaris*

Results and Discussions: Plasma treatment on fruiting body production, cordycepin, and adenosine



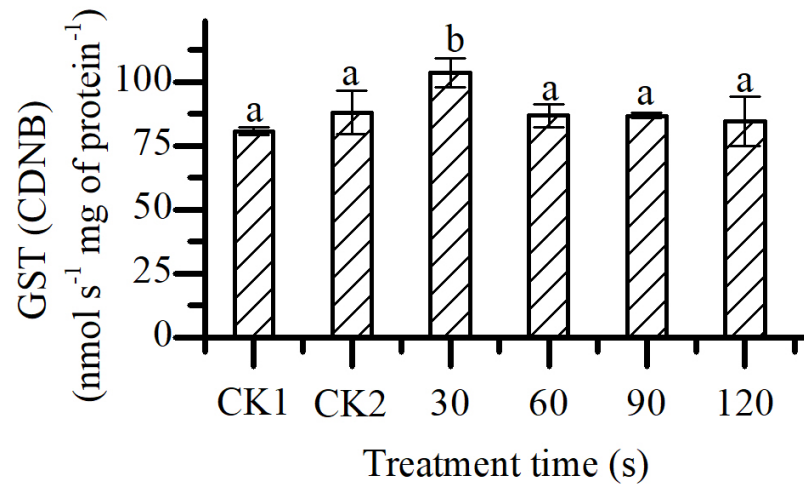
Fruiting body production during cultivation



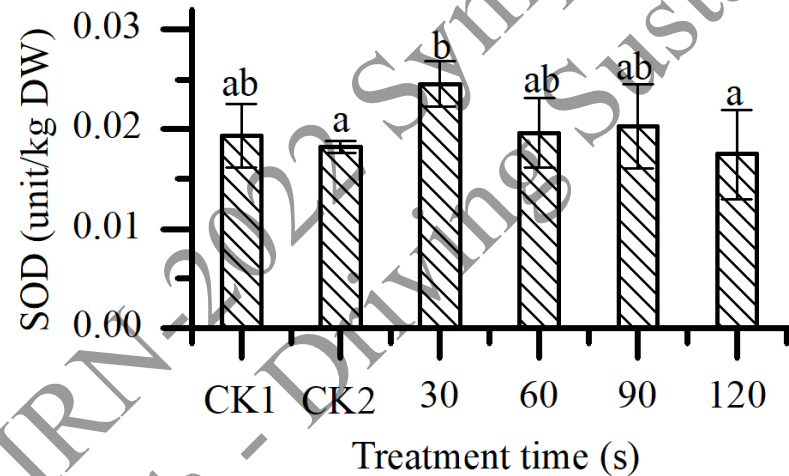
(a) control (b) 30 s Plasma treatment

Results and Discussions: Antioxidant enzymes and lipid peroxidation

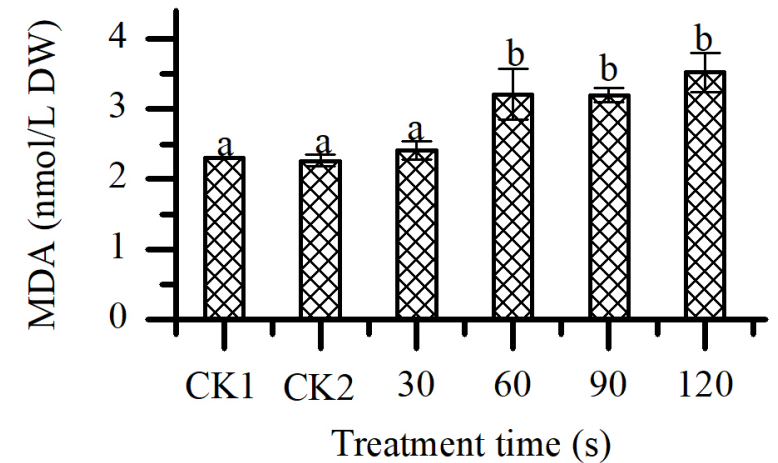
Glutathione S-transferase



Superoxide dismutase



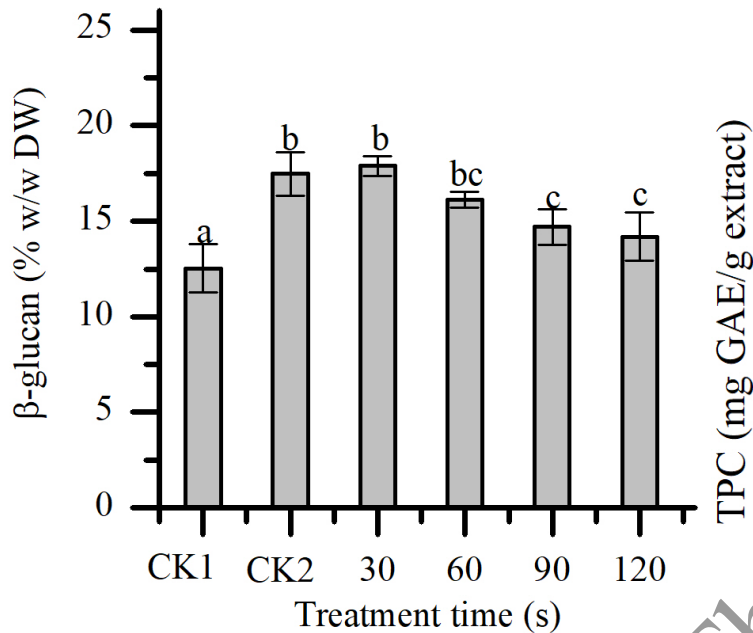
Malondialdehyde



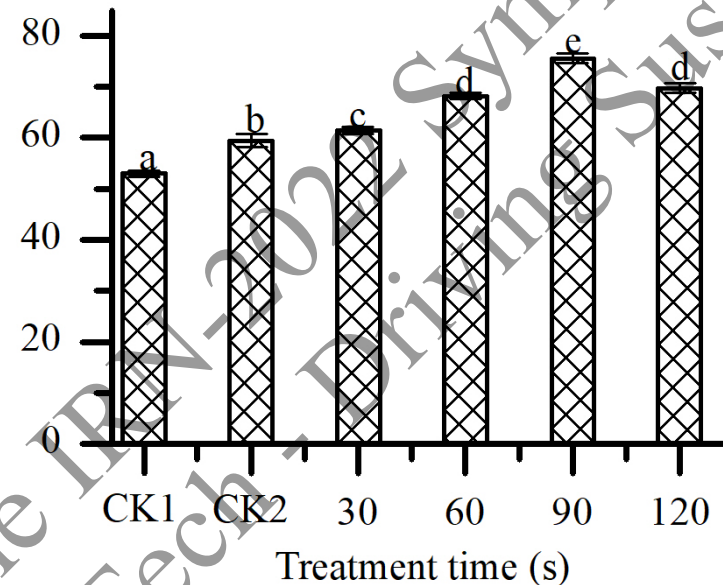
CK1: Control of white *C. militaris*, CK2: Control of blended *C. militaris*.

Results and Discussions: β -glucan and bioactive compounds

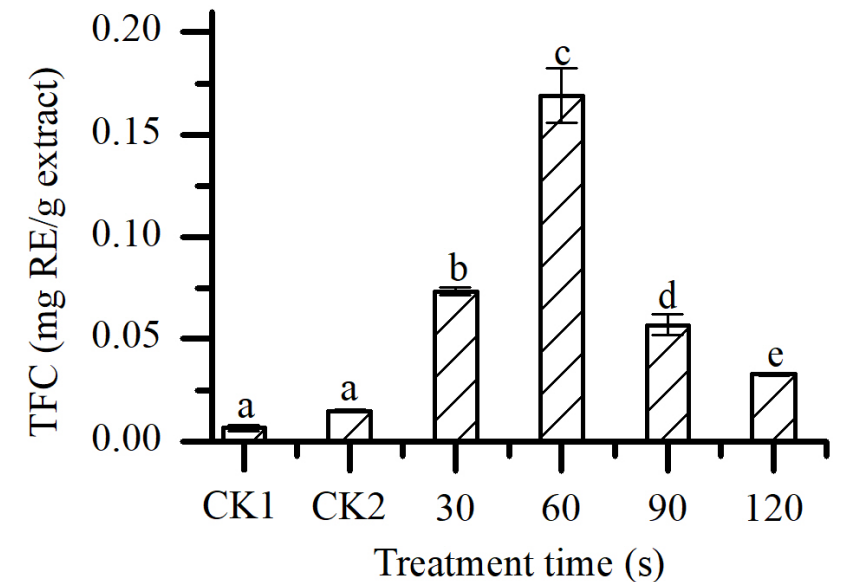
β -glucan



Total phenolic content



Total flavonoid content

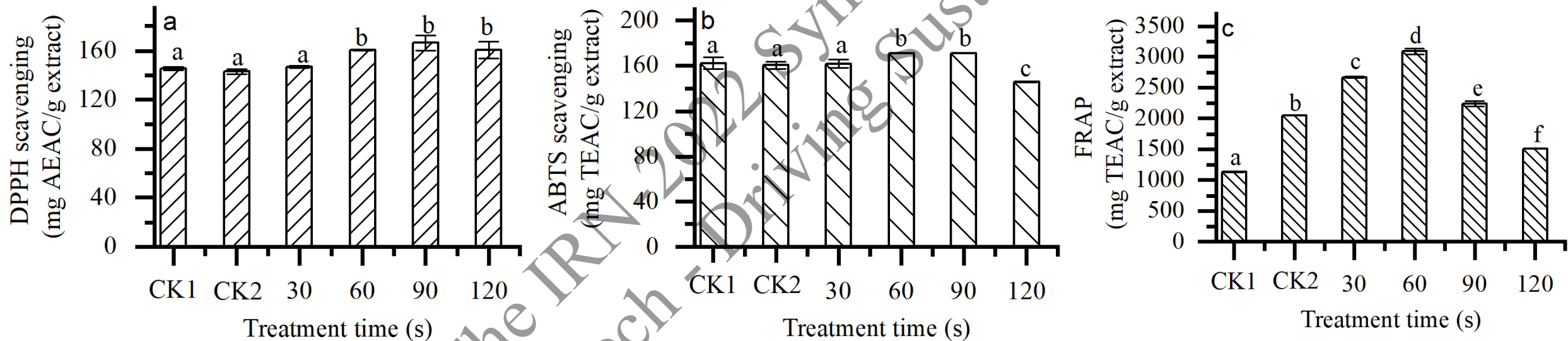


CK1: Control of white *C. militaris*, CK2: Control of blended *C. militaris*.

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Results and Discussions: Antioxidant activities

(a) DPPH and (b) ABTS free radical scavenging ability (c) Ferric-reducing antioxidant power



CK1: Control of white *C. militaris*, CK2: Control of blended *C. militaris*.

Results and Discussions: Pearson's correlation coefficients

Antioxidant activities could be governed by TPC and TFC.

Assay	DPPH scavenging activity	ABTS scavenging activity	FRAP
TPC	0.909**	0.191	-0.001
TFC	0.329	0.590*	0.851**

*Correlation is significant at $p < 0.05$ (two-tailed). **Correlation is significant at $p < 0.01$ (two-tailed).

Pearson's correlation coefficients between the contents of **bioactive compounds (TPC and TFC)** and **antioxidant activities (DPPH and ABTS scavenging activities, FRAP)**.

Conclusion

- Cold plasma jet was operated under a 50% duty cycle of a neon transformer and a fixed airflow rate of 5 L/min. The production of reactive species NO, \cdot OH, and O was measured in the gas phase.
- The interaction of these reactive species with the H₂O molecule led to the generation of **nitrite and nitrate** in the liquid media
- Fruiting body production in blended *C. militaris* was improved in response to Plasma treatments as determined by increased cordycepin and antioxidant enzyme activities and the reduced MDA levels
- Bioactive compounds (TPC and TFC) and antioxidant activities in blended *C. militaris* fruiting bodies were significantly increased in response to cold plasma treatments.

Research team



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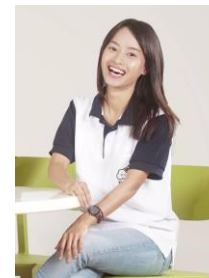
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Thank you for your attention

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