1 <u>Mini Review Pape</u>																																								M	lir	ii.	Re	vie	W I	Pa	p	? r
2 Exploitation of cold plasma technology for seed germinatio										F	Ξx	K]	p)]	.0	it	a	tio	n	OÍ	f (C	ol	ld	l]	p]	la	SI	m	a	t	e	c	hı	n	ble	og	y	fo	r	se	ed	g	ern	nin	nat	tio	n
3 enhancemen																																										(en	hai	ıce	em	lei	nt
4 ABSTRACT																										A	AB	S	Tl	R/	40	C	Г															
5 Cold plasma is an emerging nonthermal technology primarily used for microbial disinfection a	old	Colo	Co	Co	old	l pl	las	sm	m	a i	is	а	ın	1 (er	ne	erg	jing	g ne	on	ith	er	m	ıa	.1 t	tec	chı	no	olo	g	y]	pr	in	na	ril	y	use	d t	for	m	icr	obi	al c	lisin	fect	tior	n a	nd

surface modification. The principle of plasma surface modification is exploited in food and 6 7 agriculture for enhance of seed germination and other reasons. The aim of the present review is to givesome insights on cold plasma technology exploitation for enhancement of seed 8 germination. The seed germination rate can be increased on application of cold plasma by both 9 direct and indirect treatments. Recently, the indirect treatment through the application of plasma 10 activated water (PAW) has drawn some attentions. The formation of reactive oxygen species and 11 reactive nitrogen species in the plasma are mainly responsible for increase in seed germination 12 13 rate. Of all those reactive species formed in the PAW, the nitrate ions severe as the fertilizer and NO radical breakdown dormancy which enhanced the seed germination rate. The synergistic 14 effect of cold plasma can replace the traditional seed disinfection solutions and chemical seed 15 germination enhancers. 16

17 Key words: Seed; germination; cold plasma; plasma activated water; reactive species; plant18 yield

19 1. INTRODUCTION

20 The present human population statistic says that the population of the earth reached to 21 about 7.6 billion. It is insufficient for the total cultivable land to meet the demand of increasing population consumption. In order to meet this demand, the only way is to increase the crop 22 23 yields. Enhancing the seed germination rate and a draught resistant seed is the best way to 24 improve crop production. The most commonly used methods to enhance the seed germination 25 are the chemical methods (agrochemicals, fungicides, insecticides and hormones). But these types of treatments leave chemical residues which are harmful to the human health and 26 27 environment. Therefore, it is necessary to develop new alternative technologies to enhance seed

germination to keep up with pace of growing population. [1] reported that the nonthermal and
electromagnetic technologies have been successfully applied to enhance the seed germination.
Cold plasma is one of such technologies recently drawn interest in food and agriculture sector.

Plasma is known as the fourth state of matter, charged gas with strong electrostatic 31 interactions. Plasma consists of neutral and excited atoms, free radicals, negative and positive 32 ions, UV photons with net electric charge zero. [2] reported that the plasma is divided into high 33 34 temperature (local thermal equilibrium) and low temperature (non-local thermal equilibrium). The cold plasma contains low temperature particles like neutral molecules, atomic species and 35 relatively high temperature electrons because of this the plasma is cold and does not affect the 36 sensitive materials which comes into contact [3]. [4] Stated that cold plasma treatment is thought 37 38 to be a fast, economic and pollution-free method to improve seed performance, plant growth and ultimately plant yield. 39

The cold plasma treatment of seeds has a synergistic effects i.e. it acts as antimicrobial agent (ability to kill wide range of microbes) and enhance the seed germination and plant growth. Till date there are many reviews on the antimicrobial effects of cold plasma treatment but only few reviews on the seed germination enhancement. The aim of the present review is to give some insights on the seed germination enhancement by the cold plasma treatment.

45 2. EFFECT OF COLD PLASMA ON SEED GERMINATION

[5] reported that the seedling growth during germination involves two key steps 1) primary cell 46 elongation of the axial part of the embryo, and 2) simultaneous or delayed cell division in the 47 radicle meristem. [6] reported that the seed germination is initiation of embryo breaking the 48 dormant stage and always start with imbibition of water. Seed germination activity involves 49 50 several physiological and biochemical changes such as protein synthesis, enzyme activation and 51 starch metabolisms [7]. The authors have also reported that the seed germination always hindered by seed dormancy factors which is undesirable process. The cold plasma can be applied 52 by two different ways 1) direct treatment of seeds, 2) indirectly treating the seeds with plasma 53 54 activated water (PAW) or plasma acid.

55 **2.1 Direct treatment**

56 In direct treatment method the seeds are directly placed in between the electrodes or placed 57 under the plasma regime like in plasma jets. Direct exposure of chickpea seeds to atmospheric

cold air plasma for 5 min by [8] observed an overall increase in seed germination by 89.2%. The 58 authors have reported that the increase in the seed conductivity and seed roughness after the 59 plasma treatment is the main reason for enhancement. The increase in seed roughness or etching 60 caused by bombardment of reactive species may be the reason for increase in hydrophilicity of 61 seeds. [9] reported that the radio frequency low pressure plasma treatment of brown rice 62 increased the hydrophilicity. Plasma etching is the removal of volatile substrates removed by 63 64 chemical reactions and/or physical means (ion bombardment) and which are carried away in the gas stream. An investigation carried out by [10] on the wettability of seeds using cold air plasma 65 decreased the contact angle was from 115° to 0° . The plasma treatment resulted in complete 66 hydrophilicity of seeds. The water imbibition of plasma treated seeds was increased by 30% after 67 the 12 h of germination. 68

[11] exposed barely seeds to both continuous and pulsed glow discharge plasmas, with a pulse repetition rate of 0.5 Hz and pulse duration of150–200 ms in air under 0.1–0.2 Pa. The authors reported that the number of germinated seeds after the exposure was increased more than 27% after the 5th day of germination. Low pressure plasma treatment of mung beans with a radio frequency (13.56 MHz) significantly enhanced the germination rate by 36.2%, radicle root length by 20% and conductivity of seeds by 102% when compared to the untreated samples [12].

A detailed study conducted by [7] on germination of coriander seeds using nitric oxide 75 76 (NO) gas produced from microwave plasma torch and explained all the possible ways for increase in seed germination rate. The authors have observed 91-97% germination of seeds after 77 treatment whereas only 60% in the case of control/untreated seeds. The treated seeds showed 78 79 longer radicles and stems length compared to control seeds. The NO formed in the plasma serve 80 as a signaling pathway, triggers activation of several biological processes and a crucial regulator for cellular activation. The authors finally concluded their argument that the NO formed in the 81 plasma is responsible for seed germination enhancement. A similar explanation was given by 82 [13] on the synergistic effect of formation of endogenous NO radicals and breakdown of seed 83 dormancy resulted in increase in higher germination rates. 84



Figure 1 Seedling growth of radio frequency cold plasma treated mung beans after 24 h ofgermination [12]

[14] reported that the oxygen plasma treatment of corn seeds increased the germination 88 89 rate and higher yields. Another study conducted by [15] on effect of corona discharge plasma jet on sprouting of rapeseeds. Exposure of rapeseeds for 1 min increased the germination rate by 90 91 7.7% compared to untreated seeds. The authors have reported that exposure of seeds resulted in scarification; formation of deep longitudinal cracks on the seed surface, increases in surface are 92 93 attributing to increase in surface energy. [16] studied the effect of cold plasma on seed germination of oat corns and wheat at 500 W at different time durations. The authors have 94 observed that plasma treatment did not affect the germination of oats but there is slight increase 95 in germination rate in wheat. Similar increase in the germination rate of rice was observed after 96 the treatment with static magnetic fields [17]. Table 1 report on the different seeds enhancement 97 caused by cold plasma. 98

99 **2.2 Indirect treatment**

In this type of treatments the seeds are treated with plasma activated water (PAW). The PAW is generated by application of cold plasma on the water surface or underneath water using different plasma sources. [17] reported that based on the working gas, discharge type and chemical composition of plasma several reactions are initiated in the water resulting in primary and secondary reactive species changing the physico-chemical properties of water. The important reactive species formed in the PAW are reactive oxygen species (ROS) like atomic oxygen, singlet oxygen, superoxide, hydroxyl radicals and reactive nitrogen species (RNS) like

peroxynitrite, nitric oxide, nitrates and nitrite ions. The formation of these reactive species
attributes some important change in properties of water are pH, oxidation-reduction potential,
conductivity, H₂O₂concentration, nitrites and nitrates concentration.

[19] conducted a study on underwater electric front type discharge to generate PAW and 110 treatment of PAW on rye seeds. The rye seeds treatment with PAW for 5 min increased the 111 germinability by 50% and the number of seeds germinated. Along with the hormones, the ROS 112 113 and NO radicals participate in several signaling pathways involved in the seed germination [20]. The studies related to germination suggested that the ROS including superoxide, hydroxyl 114 radicals, hydrogen peroxide and atomic oxygen are responsible for seed germination [21]. An 115 investigation carried out by [6] on the seed germination of spinach observed a slight increase in 116 germination rate after treating with DBD N₂ plasma. The authors have also reported increase in 117 level of GA₃ hormone and mRNA expression of an amylolytic enzyme related gene expression 118 in seeds. [5] reported that the reactive nitrogen species (RNS) particularly NO can break the seed 119 dormancy and enhance the seed germination. 120

A study conducted by [22] on the effect of PAW (generated using cylindrical double 121 122 DBD (Cyl-DBD) reactor and tap water on seed germination. The authors have observed 60% and 100% germination rate when seeds treated with PAW-15 and PAW-30 respectively along with 123 improved seedling growth whereas only 40% with tap water. They have stated that the increase 124 in nitrate ion concentration is the main reason for obtaining higher germination rates. [23] 125 126 reported that the nitrate species formed in PAW served as a fertilizer which resulted in enhanced 127 growth. The activation of tap water with the atmospheric plasma jet increased the germination rate of lentil seeds to 80% compared to 30% of untreated seeds (Figure 2). 128



129

Figure 2 Photographs of S. oleracea cultivated for 0 (upper) and 28 days (lower) under control
conditions, PAW generated for 15 and 30 min (Adapted from[23])

132 **3. Future directions**

The chemistry of cold plasma is very complex involving many chemical reactions mainly governed by the plasma parameters. A detailed studied to be carried on understanding the mechanism of breakdown of seed dormancy and enhancement of seed germination. After treatment, the toxicology studies need be carried out. A future investigation is required on the enzymatic activities taking place in the germinating seeds. Cost of the treatment and operating cost and set up cost at the large scale is still under study.

139 4. CONCLUSION

140 It can be concluded from the reports of several researchers that the seed germination rate 141 enhancement is achieved from cold plasma technology. The plasma etching or scratching effect 142 on the seed coat increased the hydrophilicity of seeds. Few authors have reported that few 143 reactive species formed in the plasma has the ability to breakdown the seed dormancy and 144 increased the seed germination rate. Cold plasma can be considered as the alternative technology 145 for enhancement of seed germination rate and plant yield.

146

147 **COMPETING INTERESTS**

148149 Authors have declared that no competing interests exist.

150

150

151 **REFERENCES**

- Filatova I, Azharonok V, Kadyrov M, Beljavsky V, Gvozdov A, Shik A, Antonuk A.
 The effect of plasma treatment of seeds of some grains and legumes on their sowing
 quality and productivity. *Roman Journal of Physics*. 2011; 56, 139–143.
- Bogaerts, A., Neyts, E., Gijbels, R. and Van der Mullen, J. Gas discharge plasmas and
 their applications. *Spectrochimica Acta Part B:* At. Spectrosc., 2002; 57(4): 609-658.
- Thirumdas R, Trimukhe A, Deshmukh R R, Annapure U S. Functional and rheological
 properties of cold plasma treated rice starch. Carbohydr. Polym. 2017; 157: 1723-1731.
- Ling L, Jiafeng J, Jiangang Li, Minchong S, Xin H, Hanliang S, Yuanhua D. Effects
 of cold plasma treatment on seed germination and seedling growth of soybean. Scient
 Rep. 2014; 4, 5859.
- 5. Šírová J, Sedlářová M, Piterková J, Luhová L, Petřivalský M.The role of nitric oxide in
 the germination of plant seeds and pollen. Plant Sci. 2011; *181*(5): 560-572.
- 6. Ji SH, Choi KH, Pengkit A, Im J S, Kim, JS, Kim YH, Park G. Effects of high voltage
 nanosecond pulsed plasma and micro DBD plasma on seed germination, growth
 development and physiological activities in spinach. Arch. Biochem. Biophys, 2016; 605,
 117-128.
- Ji S, H, Kim T, Panngom K, Hong Y J, Pengkit A, Park, D H, Uhm H S. Assessment of
 the Effects of Nitrogen Plasma and Plasma-Generated Nitric Oxide on Early
 Development of Coriandumsativum. Plasma Processes Poly. 2015; 12(10): 1164-1173.
- Mitra A, Li YF, Klämpfl TG, Shimizu T, Jeon J, Morfill G E and Zimmermann J L.
 Inactivation of surface-borne microorganisms and increased germination of seed
 specimen by cold atmospheric plasma. Fd Bioprocess Technol. 2014; 7(3): 645-653.
- Thirumdas, R., Saragapani, C., Ajinkya, M. T., Deshmukh, R. R. and Annapure, U. S.
 Influence of low pressure cold plasma on cooking and textural properties of brown
 rice. Innov. Food Sci.& Emerg. Technol. 2016; 37: 53-60.

177	10. Bormashenko, E., Grynyov, R., Bormashenko, Y.and Drori, E Cold radiofrequency
178	plasma treatment modifies wettability and germination speed of plant seeds. Scient
179	Rep.2012; 2: 741.
180	11. Dobrin, D, Magureanu M, Mandache NB, and Ionita M D.The effect of non-thermal
181	plasma treatment on wheat germination and early growth. Innov. Food Sci. Emerg.
182	Technol.2015: 29, 255-260.
183	12. Sadhu S., Rohit T., Deshmukh.R.R. Annapure .U.S., Influence of cold plasma on the
184	enzymatic activity in germinating mung beans (Vigna radiate). LWT - Food Sci.
185 186	& Tech. 2017: 78, 97-104.
197	plasma activated tap water demineralized water and liquid fertilizer RSC Adv
107	plasma activated tap water, demineralized water and inquid fertilizer. $KSC = Aav$.
100	14 Violleou E Hadiaba K Albet I Cazalis P and Sural O Effect of ovidative
100	treatment on corn seed cormination kinetics. Ozone Sci. Eng. 2008: 20(6): 418-422
190	15 Puligundle, P. Kim, I.W., Mak C. Effect of corona discharge plasma ist treatment on
191	15. Pungundia P, Kim J W, Mok C. Effect of corona discharge plasma jet treatment on
192	decontamination and sprouting of rapeseed (Brassica hapus L.) seeds. Food Control.
193	2017; 71: 370-382.
194	16. Sera B, Spatenka P, Sery M, Vrchotova N. and Hruskova I. Influence of plasma treatment
195	on wheat and oat germination and early growth. <i>IEEE</i> Trans on Plasma Sci. 2010; 38(10):
196	2963-2968
197	17. Carbonell, M. V., Martinez, E., Amaya, J. M.Stimulation of germination in rice (Oryza
198	sativa L.) by a static magnetic field. Electro- Magnetobiol. 2000, 19(1), 121-128.
199	18. Shen J, Tian Y, Li Y, Ma R, Zhang Q, Zhang J, Fang J. Bactericidal effects against S.
200	aureus and physicochemical properties of plasma activated water stored at different
201	temperatures. Scient Rep. 2016; 6:23-29
202	19. Naumova I K, Maksimov A I, Khlyustova AV. Stimulation of the germinability of
203	seeds and germ growth under treatment with plasma-activated water. Surf. Eng. Appl.
204	Electrochem. 2011; 47(3): 263-265.
205	20. El-Maarouf-Bouteau H, and Bailly C. Oxidative signaling in seed germination and
206	dormancy. Plant Signali and Behavior. 2008; 3(3), 175-182.

- 207 21. Su L, Lan Q, Pritchard H W, Xue H. Wang X. Reactive oxygen species induced by cold
 208 stratification promote germination of Hedysarumscoparium seeds. Plant Physiol.
 209 Biochem. 2016; 109: 406-415.
- 210 22. Sivachandiran L. and Khacef A. Enhanced seed germination and plant growth by
 211 atmospheric pressure cold air plasma: combined effect of seed and water treatment. *RSC*212 *Adv.* 2017; 7(4): 1822-1832.
- 213 23. Takaki K, Takahata J, Watanabe S, Satta N, Yamada O. Fujio T, Sasaki Y.
 214 Improvements in plant growth rate using underwater discharge. In J. Phys. *Conference*215 *Series*. 2013;418 (1): pp 01231-01240. IOP Publishing.
- 24. Schnabel U, Niquet R, Krohmann U, Winter J, Schlüter O, Weltmann KD, Ehlbeck J.
 Decontamination of microbiologically contaminated specimen by direct and indirect
 plasma treatment. Plasma Processes Polym. 2012: *9*(6), 569-575.
- 219 25. Stolárik, T, Henselová M, Martinka M, Novák O, Zahoranová A, Černák M. Effect of
 220 low-temperature plasma on the structure of seeds, growth and metabolism of endogenous
 221 phytohormones in pea (Pisumsativum L.). Plasma Chem. Plasma Process. 2015; *35*(4):
 222 659-676.
- 223 26. Jiayun T, Rui H, Xiaoli Z, Ruoting Z, Weiwen C, Size Y. Effects of atmospheric
 224 pressure air plasma pretreatment on the seed germination and early growth of
 225 Andrographispaniculata. *Plasma Sci Technol*, 2014; *16*(3), 260.
- 27. Hayashi N, Ono R, Shiratani M, Yonesu A. Antioxidative activity and growth regulation
 of Brassicaceae induced by oxygen radical irradiation. Jpn. J. Appl. Phys. 2015; *54*(6S2),
 06GD01.
- 229 28. Meiqiang Y, Mingjing H, Buzhou M. Tengcai M. Stimulating effects of seed treatment
 230 by magnetized plasma on tomato growth and yield. Plasma Sci Technol, 2005; 7(6):
 231 3143.
- 232 29. Ling L, Jiangang L, Minchong S, Chunlei Z, Yuanhua D. Cold plasma treatment
 233 enhances oilseed rape seed germination under drought stress. Scient Rep. 2015; 5: 130234 139; doi: 10.1038/srep13033.
- 30. Ling L, Jiangang L, Minchong S, Chunlei Z, Yuanhua D. Cold plasma treatment
 enhances oilseed rape seed germination under drought stress. Scient Rep. 2014; 5: 130139; doi: 10.1038/srep13033.

Table 1 Key findings of different studies conducted on the seed germination rate using cold

239 plasma technology

Seeds	Plasma source	Feed gas	Key findings	References
Rapeseed	DBD plasma	Argon	No change in the germination rate. Increase in seed viability was observed	[24]
Pea	Diffuse coplanar surface barrier discharge	Air	Increase in germination percentage, root length, shoot length, seed vigor	[25]
Herbaceous plant	DBD plasma	Air	Permeability of the seeds was improved significantly, acceleration of seed germination and seedling emergence	[26]
Radish sprout	atmospheric pressure plasma torch	Oxygen	Enhancement of the germination and lengths of the stem and root of plants are observed after seeding	[27]
Tomato	Arc discharge Plasma	Air	In pot experiments the yield is increased by 20.7%. Sprouting rate after the treatment is 32.75%, whereas the untreated was only 4.75%	[28]
Wheat	Atmospheric pressure surface discharge	Air	Plasma had little effect on germination rate but the distribution of roots was shifted towards higher lengths as compared to untreated samples	[11]
Rapeseed	Radio frequency discharge	Helium	Significant improvement in germination rate by 6.25% in drought sensitive variety, and 4.44% in drought tolerant variety	[29]
Soybean	Radio frequency discharge	Helium	Germination and vigor indices significantly increased by 14.66% and 63.33%, respectively. Water uptake improved by 14.03%	[30]
Mung beans	Radio frequency capacitively coupled plasma	Air	Increase in following parameters- germination rate by 36.2%, radicle root length by 20% and conductivity of seeds by 102%	[12]
Coriander seeds	DBD	Nitrogen	After 7 days of germination there is 90% of seed germination compared to 40-60% for control seeds	[6]