

Original Research Article

<https://doi.org/10.20546/ijcmas.2021.1001.172>

Cold Plasma Treatment for the Control of *Alternaria solani* causing Early Blight of Tomato

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ABSTRACT

Keywords

Cold plasma, Radio frequency, Tomato, *Alternariasolani*, Early blight

Article Info

Accepted:
12 December 2020
Available Online:
10 January 2021

Plasma (fourth state of matter) is ionized gas composed of positive and negative ions, electrons, neutrals, molecules, photons and UV rays. Plasma seed treatment is an emerging new technique for seed quality control. Effect of cold plasma treatment on the control of *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of tomato (*Solanum lycopersicum* L.) cultivar PKM 1 was studied. The seeds were exposed to various durations of non-thermal (cold plasma) atmospheric air pressure plasma treatment using radio frequency glow discharge technique at Institute for Plasma Research, Gujarat, India. The seeds pre-inoculated with *A.solani* were plasma treated with power of 20 W and 40 W for varied durations of 0, 5, 10, 15, 20, 25 and 30 minutes. Plasma treatment significantly reduced pathogen incidence and increased both germination and plant growth in comparison with the control. Experimental results indicated that all the treatments were significantly different and the seeds treated with 40 W for 30 minutes showed minimum percent disease incidence (0.0 %) with highest percent disease inhibition over control (100 %). It also recorded the highest plant biometrics viz., germination (96 %), root length (14.41 cm), shoot length (7.56 cm) and vigour index (2109).

Introduction

Plasma, the fourth state of matter, is an ionized gas. In 1929, Tonks and Langmuir coined the term 'Plasma'. It composed of positive and negative ions, electrons, neutrals, molecules, photons and UV radiation. Non-thermal plasma is characterized by low energy of its heavy particles (ions, molecules), which means it does not damage

thermally sensitive material, but its electrons reach sufficient energy to participate in plasma-chemical reactions. Non-thermal atmospheric pressure plasma treatment is a modern biotechnological method that may be used for the decontamination of various surfaces of living tissue due to its highly reactive composition, it can treat surfaces without the need for invasive chemicals (Brelles, 2012 and Baier *et al.*, 2014). The

problem of poor or slow germination can be solved through many techniques and one of them is plasma treatment. Plasma treatment has become an important factor widely used in biotechnology, medicine & food industry (Padureanu, 2012). Dry seed treatment like plasma treatment is employed to increase the seed coat permeability without increasing the moisture content of seed. Plasma seed treatment method is a physio-chemical method which has the potential to improve germination, increase the yield and kill fungal spores which is present on the seed coat (Zhou *et al.*, 2012). Plasma treatment has been successfully applied in agriculture for seed quality improvement, seed enhancement and pathogenic micro-organisms inactivation (Filatova *et al.*, 2013). Crop yields are improved by treating the seeds in a low temperature plasma discharge generated between spaced electrodes connected to a source of high frequency electrical power (Krapivina *et al.*, 1994). Cold plasma treatment is a cost effective method that has been effectively used in seed technology because it is fast economic and pollution free method to improve the seed performance, it decontaminates the pathogens from seeds, no loss of seed quality and a quick treatment with no side effects. Tomato (*Solanum lycopersicum* Mill.) is one of the most important vegetables. The fungus *Alternaria solani* (Ellis and Martin) Jones and Grout is the causal agent of early blight disease, is a major pathogen of tomato causing considerable yield loss. Based on the available information cold plasma treatment on disinfection, germination and vigour of tomato cultivar PKM 1 seeds was studied.

Materials and Methods

Effect of cold plasma treatment on the control of *Alternaria solani* (Ellis and Martin) Jones and Grout causing Early blight of tomato (*Solanum lycopersicum* L.) cultivar PKM 1

was studied by following the below methodologies.

Isolation of pathogen and Pathogenicity test

The pure culture of *Alternaria solani* was obtained by single spore isolation method and sub culture was used for pathogenicity test by following Koch's postulate. The pathogenicity test was carried by pre-inoculation with spore suspension and homogenized mycelial bits of *A. solani* on foliage of 30 days old plants of PKM 1 cultivar of tomato. After inoculation, the symptoms appeared on inoculated leaves as brown, oval or angular necrotic spots with concentric rings and surrounded by a border of yellow host tissue. The fungus was re-isolated and purified culture from these artificially infected leaves was similar to that of original culture. The plants which were not inoculated with the fungal spore suspension did not show any symptoms of the disease. Thus pathogenicity on tomato was confirmed (Prathima *et al.*, 2018)

Seed inoculation

Tomato seeds were immersed in a solution of 10% commercial bleach with Tween20 (1 drop/100 mL of solution) for 15 min, followed by 2 rinses with sterile distilled water for 5 minutes. After that, they were immersed in hydrogen peroxide (33%) for 10 min and rinsed three times with sterile distilled water for 10 minutes. Later, seeds were stirred in water for 24 h and then air-dried in a laminar flow cabin for at least 2h. Spores of *A. solani* were obtained by scraping a 7-day-old culture of a fungus colony growing on PDA at 25 °C, adding 0.5% KCl + 1 drop of Tween20 and filtered through two layers of cheese cloth. The concentration was adjusted to give a spore suspension of 10^3 spores/ μ L. Seeds were immersed and stirred

in this spore suspension for 30 s and then air dried used for plasma treatment (Evira-Recuenco, *et al.*, 2015)

Plasma device and Plasma treatment

Non-thermal (cold plasma) atmospheric air pressure plasma treatment using radio frequency glow discharge technique at Institute for Plasma Research, Gujarat, India was used at a base pressure of 0.05 m bar, operating pressure of 0.2 m bar, voltage of 500 V, current of 0.2 A and power of 20 W and 40 W for varied durations of 0, 5, 10, 15, 20, 25 and 30 minutes. Tomato seeds were spread in glass petri dish (150 mm in diameter), and they were kept into the plasma apparatus. Seeds were exposed to inductive plasma treatment with RF discharge. Mean while, without plasma treatment, control seeds were also exposed to the same vacuum as the treated seeds (Cherry *et al.*, 2018).

Standard Blotter Method

Detection of seed borne fungi in seed samples was done by following ISTA procedures. In this method, three layers of blotter paper was soaked in sterilized water and placed in the petri plates. 100 seeds were sterilized in 0.2% Sodium hypochlorite solution for 2 to 3 minutes and seeds taken randomly from each sample and were placed in petri plates and incubated for seven days in the laboratory under alternating cycles of 12 hrs light and 12 hrs darkness. The incubated seeds were examined under stereo binocular microscope to ascertain the presence of fungi (ISTA, 1993).

Germination (%)

Four replicates of 100 seeds were uniformly placed on standard germination paper roll-towel medium and kept in germination room maintained at $25 \pm 2^\circ\text{C}$ and 90 ± 2 per cent

relative humidity. After 14 days, the seedlings were evaluated as total number of normal seedlings and germination as percentage (ISTA, 1993).

Root length and Shoot length

On fourteenth day, ten normal seedlings per replication from roll towel medium were carefully removed at random from each treatment. The root length was measured from the base to the top of the primary root and the shoot length was measured from the base of the shoot to tip of primary leaf and the mean value was calculated and expressed in cm (ISTA, 1993).

Vigour index

The Vigour index was calculated and compared by adopting the following formula and expressed as whole number (Abdul-Baki and Anderson, 1973).

Statistical analysis

The data obtained from various experiments were analysed statistically by adopting the procedure described by Panse and Sukhatme (1985). The laboratory experiments were laid out in completely randomized design (CRD). The data recorded on per cent values were arc-sine transformed before analysis and the critical differences (CD) were calculated at 5 per cent probability level.

Results and Discussion

Standard blotter method for detection of seed borne fungi in seed samples was done by following ISTA procedures. The results indicate that all the treatments were significantly different with varied level of pathogen infection. Among the different cold plasma treatments tomato seeds treated with 40 W performed better than 20 W and the

seeds treated with 40 W for 30 min recorded the minimum percent disease incidence (0.0 %) with highest percent disease inhibition over control (100 %) (Table 1). It also recorded the highest plant biometrics viz., germination (96 %), root length (14.41 cm),

shoot length (7.56 cm) and vigour index (2109). Control recorded the minimum plant biometrics viz., germination (61 %), root length (12.92 cm), shoot length (6.17 cm) and vigour index (1164) (Table 2).

Table.1 Effect of Cold plasma treatment on tomato cv. PKM 1 seeds inoculated with *Alternaria solani*

Cold Plasma	20 W		40 W	
	SBM % infection (%)	Per cent Inhibition over control (%)	SBM % infection (%)	Per cent Inhibition over control (%)
5 minutes	82.50	17.50	39.23	60.77
10 minutes	77.87	22.13	31.14	68.86
15 minutes	71.29	28.71	23.72	76.28
20 minutes	60.02	39.98	17.45	82.55
25 minutes	53.37	46.63	9.26	90.74
30 minutes	45.34	54.66	0.00	100.00
Control	100.00	–	100.00	–
SEd	2.01	0.75	0.41	1.43
C D (P = 0.05)	4.30	1.64	0.87	3.12

*SBM – Standard Blotter Method

Table.2 Effect of Cold plasma treatment on biometrics of tomato cv. PKM 1 seeds inoculated with *Alternaria solani*

Cold Plasma	20 W				40 W			
	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index
5 minutes	71	6.25	13.05	1370	82	6.99	13.96	1718
10 minutes	74	6.31	13.27	1449	83	7.15	14.01	1756
15 minutes	76	6.59	13.52	1528	88	7.23	14.17	1883
20 minutes	77	6.71	13.63	1566	91	7.42	14.27	1974
25 minutes	79	6.84	13.78	1629	92	7.45	14.38	2008
30 minutes	82	6.97	13.94	1715	96	7.56	14.41	2109
Control	61	6.17	12.92	1164	61	6.17	12.92	1164
SEd	1.33	0.14	0.24	36.94	1.60	0.17	0.24	26.66
C D (P = 0.05)	2.85	0.31	0.52	79.23	3.44	0.36	0.51	57.17

Non-thermal plasma generated using air DBD significantly reduced fungal inoculation of *Gibberella fujikuroi* from rice seed surface (Yo *et al.*, 2014) and total growth inhibition of *Fusarium nivale* and *F. culmorum* was observed (Zahoranova *et al.*, 2016). The seeds of some plant species usually germinate well after non-thermal plasma treatment, e.g. some grains (Hertwig *et al.*, 2017 and Dubinov *et al.*, 2000); *Chenopodium album* agg. and *Papaver somniferum* (Sera *et al.*, 2009 and 2013); *Zea mays* (Henselova *et al.*, 2012); *Pisum sativum* (Stolarik *et al.*, 2015); *Brassica napus* (Puligundla *et al.*, 2017); *Raphanus sativus* (Mihai *et al.*, 2014). In addition to improving seed germination by breaking the dormancy, cold plasma treatment has previously been reported to inactivate seed-borne pathogenic microorganisms (Selcuk *et al.*, 2008 and Schnabel *et al.*, 2012). Several studies have shown that low-temperature plasma can inhibit the growth of food pathogens (Dasan *et al.*, 2016 and Butscher *et al.*, 2016) in addition to phytopathogenic fungi (Zhang *et al.*, 2014). Mitra *et al.*, 2014, found that a significant reduction of the seed-borne microbial contamination of non-thermal plasma treatment in chickpea seeds (*Cicer arietinum*). The increased plant growth is attributed to increased auxin metabolism, cell wall plasticity and permeability of cell membrane, increasing photosynthates and rapid cell elongation (Sadavarte and Gupta, 1963).

Not only the chemical structure but also the roughness of the surface is affected by the plasma treatment, which can change the wettability of the surface (Dubinov *et al.*, 2000). Reason may be removal of thin lipid layer due to exposure of seeds to plasma treatment, which makes the seed hydrophilic thereby improving the germination and seed quality (Sera *et al.*, 2010). It is a cost effective and ecologically sustainable method.

In conclusion the fast acting, economically viable and environmentally friendly techniques are needed to control plant disease during agricultural activities. Cold plasma technology is a new promising technology for phytopathogen control and has many advantages over traditional methods *viz.*, activation of endogenous substances in seeds, rejuvenation, promotion of plant growth and maximization of yield.

Acknowledgements

The authors thank Institute for Plasma Research, Gandhinagar, Gujarat, India for providing all the facilities to perform the study.

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How to cite this article:

Ragupathi, K. P., P. R. Renganayaki, S. Sundareswaran, Chetan Jariwala, S. Mohan Kumar and Kamalakannan, A. 2021. Cold Plasma Treatment for the Control of *Alternaria solani* causing Early Blight of Tomato. *Int.J.Curr.Microbiol.App.Sci.* 10(01): 1450-1456.
doi: <https://doi.org/10.20546/ijcmas.2021.1001.172>