

## Effect of Different Concentrations of the Disinfectant, Stabilized Chlorine Dioxide (Sodium Chlorite) on Cannabis Plants and their Cannabinoids Content

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**Abstract:** The effect of different concentrations of stabilized chlorine dioxide (sodium chlorite), a horticultural and agricultural disinfectant, was evaluated on cannabis plants grown in pots under controlled environmental conditions. Three consecutive applications (one application per week) of various dilutions (2000 ppm, 3000 ppm and 4000 ppm) were sprayed directly on the leaves of the plants. These plants were closely observed for any stress caused by the different concentrations of the product. Our results show that cannabis plants were unaffected by the treatment. No sign of stress were observed after three weeks of spray. A set of plants, served as control, were treated with distilled water used for dilution of the product. All the plants (treated and control) were grown up to maturity, harvested, dried, analyzed and compared for cannabinoids content *i.e.* D<sup>9</sup>-Tetrahydrocannabinol (D<sup>9</sup>-THC), Cannabidiol (CBD), Tetrahydrocannabivarin (THCV), Cannabinol (CBN), Cannabigerol (CBG) and Cannabichromene (CBC). No significant difference was found in the cannabinoids content of the treated (up to 4000 ppm) and control cannabis plants.

**Key words:** Cannabis, Stabilized chlorine dioxide (sodium chlorite), Disinfection, Δ<sup>9</sup>-Tetrahydrocannabinol, Tetrahydrocannabivarin, Cannabinol, Cannabigerol, Cannabichromene.

### INTRODUCTION

Cannabis plant belongs to the family Cannabaceae. This is one of the oldest cultivated plants known in

medicine with a reported use for the treatment of a wide variety of ailments for centuries (Doyle and Spence, 1995; Cunha *et al.*, 1980;

Mechoulam and Carlini, 1978; Pertwee, 2006; Pryce and Baker, 2005). However, it was not until the last part of the 20<sup>th</sup> century when the first cannabis based therapeutic was introduced into modern medicine named Marinol®. On the other hand, cannabis is one of the well-known/most abused plant/drug worldwide. Over the last few years, growing of cannabis for medicinal or recreational purpose has become a major agricultural industry in numerous countries. However, from the growers' point of view, there are challenges in cannabis cultivation. At least 88 fungal species (McPartland 1992) and eight pathovarieties of plant pathogenic bacteria (McPartland *et al.* 2000) are reported to cause diseases in cannabis crops. Moreover, some phytopathogens are unique to cannabis (McPartland 1984). The most threatening diseases of flowering tops are caused by three ubiquitous fungi—*Botrytis cinerea* (the cause of gray mold), *Trichothecium roseum* (white mildew or pink rot), and *Alternaria alternata* (brown blight) (McPartland and McKernan, 2017). In light of these hurdles, a disease free crop is a challenge for cannabis industry and therefore, research is ongoing in search of disinfectants for a clean and harmless product.

Chlorine based compounds are used as disinfectant for horticultural and agricultural systems for seed and plant disinfection, for disinfection of working surfaces and against phyto-pathogenic microorganisms since a long time (Bashan, 1991; 1994; Bashan *et al.*, 1978; Bashan and Okon, 1981; Dubrovsky *et al.*, 1994, Alcaraz-Melendez *et al.*, 1994; Toledo *et al.*, 1995; Sharon *et al.*, 1982; Soroker *et al.*, 1984; Bashan and Platt, 1980; Brown and Schubert, 1987; Brown and Wardowski, 1985; Roberts and Reymond, 1994). Chlorination of drinking water is the most common disinfectant worldwide which is one of the primary needs for humans in their daily life (Strobel and Dieter, 1990).

Chlorine dioxide is considered one of the popular alternatives to common chloride which is reported to be less toxic with no particular odor or taste and a highly efficient disinfectant against many

bacteria, viruses and parasites (Cardoso and Imthurn 2018, Zhao, 2017; Bull, 1982; Wilson *et al.*, 1989; Dietrich *et al.*, 1992; Wondergem and Van Dijk-Looijaard, 1991; Berg *et al.*, 1986; Brown and Schubert 1987; Datnoff *et al.*, 1987; Foegeding *et al.*, 1986; Pedersen and Jahromi, 1993; Rutala *et al.*, 1991; Tanner, 1989; Wyatt and Lund, 1981, Harakeh, 1987; Taylor and Butler, 1982).

The aim of this study is to reveal whether or not a commercial stabilized chlorine dioxide (sodium chlorite) based product named SanitizeIT™, developed by Aseptic Health, LLC, has any adverse effects on the health of cannabis plants and on their cannabinoid content.

## MATERIAL AND METHODS

### Plant Material

Cuttings of high THC variety of *Cannabis sativa* L. were taken from a screened and selected high THC yielding mother plant and kept under similar climatic controlled condition for vegetative growth. These cuttings/clones were grown in 4" biodegradable jiffy pots. Well rooted healthy cuttings were transplanted in bigger pots and kept under 18 hour photoperiod to grow up to desirable vegetative stage. Well-developed vegetatively grown plants were exposed to 12 hour photoperiod for flowering and were grown up to full maturity. Throughout the lifecycle, all the plants were kept under similar, controlled environmental conditions and watered and fertilized normally in equal manner.

### Application of Stabilized Chlorine Dioxide (Sodium Chlorite)

After two weeks in the flowering light cycle, plants were treated with different concentrations of stabilized chlorine dioxide (sodium chlorite) based product developed by "Aseptic Health, LLC". The product was diluted in three different concentrations *i.e.* 2000, 3000 and 4000 ppm using distilled water.

Cannabis plants were treated with the three different concentrations (three plants sprayed with each concentration) of product once a week for three weeks. The plants were directly sprayed and monitored closely for any stress caused by application. One set of three plants were sprayed by distilled water (to serve as control) used for dilution of the product, for comparison. All the plants were grown up to full maturity then harvested, dried, analyzed and compared for their cannabinoids content *i.e.*  $\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC), Cannabidiol (CBD), Tetrahydrocannabivarin (THCV), Cannabinol (CBN), Cannabigerol (CBG) and Cannabichromene (CBC) using a gas chromatography – flame ionization detector (GC-FID) analysis using the method described by ElSohly *et al.* (2016).

## RESULTS AND DISCUSSION

Cannabis can be easily grown outdoor or indoor in a well aerated soil. Both types of cultivation practices have their own advantages and disadvantages. Growing outdoor in open environment, cannabis crop encounters comparatively less challenges in terms of fungal or bacterial diseases, since natural environment helps plants in overcoming the problems. Whereas, in a close indoor environment with a fast growing species like cannabis, any disease multiplies with higher rates. Therefore cannabis industry is progressively looking for safer and fast acting disinfectants.

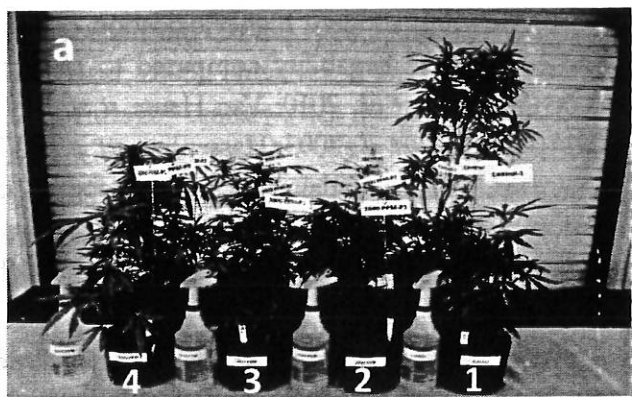
Chlorine and chlorine based compounds are highlighted as effective sanitizers. In the present day, chlorine and chlorine compounds are the most popular and effective water sanitizers to fulfill the water need of the world population. Chlorine dioxide, a derivative of Chlorine has been defined as more safe, effective and as a potential alternative to liquid chlorine as a disinfectant. The main advantage of  $\text{ClO}_2$  over chlorine is the formation of fewer types and lesser amounts of organo-halogenated

byproducts and shows a higher oxidation capacity and bactericidal capability compared to chlorine (López-Gálvez *et al.*, 2010; Van Haute *et al.*, 2015, 2017; Veschetti *et al.*, 2003; Hassenberg *et al.*, 2017). However, its bactericidal effectiveness depends on several factors, including dose, contact time, temperature, pH and organic load (Junli *et al.*, 1997; Ayyildiz *et al.*, 2009).

The stabilized  $\text{ClO}_2$  based product developed by “Aseptic Health, LLC” is claimed to be highly effective for eradicating powdery mildew a fungal disease on plants (“Aseptic Health, LLC” literature and <https://www.aseptichealth.com/>). The aims of this study were to evaluate the effect of different concentrations of stabilized  $\text{ClO}_2$  based product on the overall health and cannabinoids content of cannabis plants. Cannabis plants were sprayed with three different concentrations of the product for three weeks with one spray/week. The health of plants were assessed on two different aspects-(1) whether or not plants have gone through physical stress with increasing concentrations of product, and (2) increasing concentrations (2000, 3000 and 4000 ppm) of the product ultimately affect cannabinoids concentration of plants.

Our results show that after three weeks of spray, plants were unaffected by stabilized  $\text{ClO}_2$ . No sign of stress was evident on plants even with the application of highest concentration (4000 ppm) of product (Fig. 1).

On maturity, both sets of plants (treated and control) were individually harvested, dried and analyzed for cannabinoids content (Fig. 2a to 2e). Our results show that no significant variation was found in the cannabinoids content of plants treated with the ‘stabilized  $\text{ClO}_2$  product’ up to the highest concentration (4000 ppm) as compared to control plants. Since ‘high THC yielding variety’ of cannabis plants were chosen for the study, CBD content was found lower than limit of quantitation ( $< 0.05\%$  w/w).  $\Delta^9$ -tetrahydrocannabinol content was

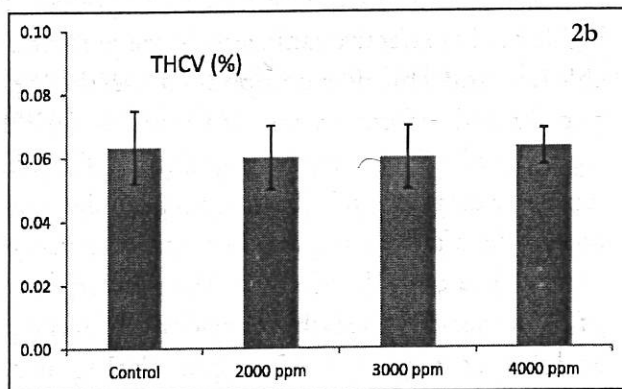
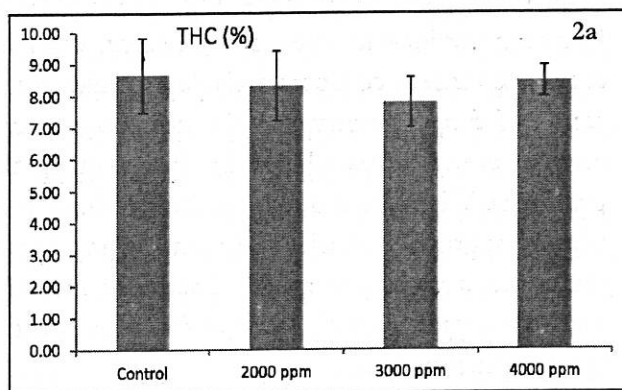


**Fig. 1:** Effect of different ‘stabilized chlorine dioxide (sodium chlorite)’ concentrations on cannabis plants. Three applications of various dilutions were performed weekly. (a) Day 0, before first spray, (b) week after first spray, before second spray and (c) two weeks after third spray. (1) Control (distilled water), (2) 2000 ppm ClO<sub>2</sub>, (3) 3000 ppm ClO<sub>2</sub> and (4) 4000 ppm ClO<sub>2</sub>.

8.67±1.17% in control plants whereas, it was 8.30±1.09, 7.78±0.80 and 8.45±0.50%, respectively in plants sprayed with 2000, 3000 and 4000 ppm concentrations of the product (Fig. 2a). The effect

of increasing concentrations of ClO<sub>2</sub> on THC, CBC, CBG and CBN content is shown in Fig. 2b, 2c, 2d and 2e, respectively. Tetrahydrocannabinol (THC), CBN, CBG and CBC content on control plants were 0.06±0.01, 0.23±0.03, 0.14±0.03 and 0.07±0.01% respectively. Whereas, THC, CBN, CBG and CBC content was 0.06±0.01, 0.26±0.05, 0.10±0.03 and 0.06±0.01% in plants sprayed with 2000ppm; 0.06±0.01, 0.32±0.07, 0.13±0.02 and 0.06±0.01% in plants sprayed with 3000ppm and, 0.06±0.01, 0.27±0.04, 0.15±0.03 and 0.06±0.01% in the group of plants sprayed with 4000ppm, respectively.

These results show no noticeable morpho-physiological changes and also no significant changes in cannabinoids content in the treated plants up to highest concentration of ClO<sub>2</sub> applied in this study, as compared to the controlled plants. Within the limits of this study, it can be concluded that application of ClO<sub>2</sub> is found to be safe for plants’ health up to 4000ppm, if applied on cannabis plants.



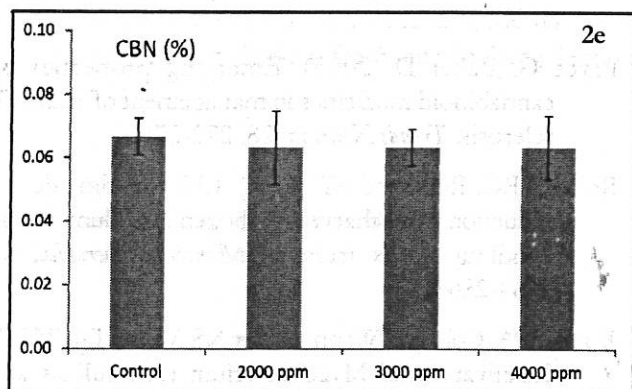
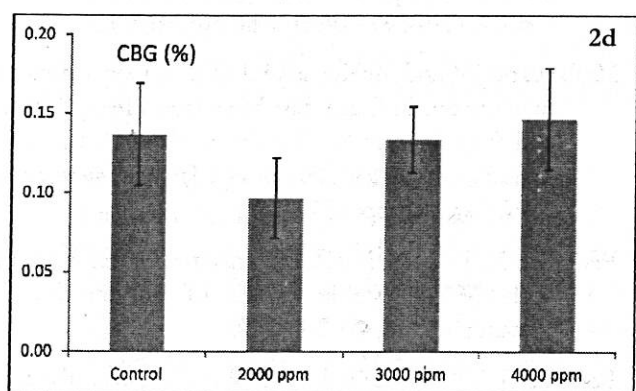
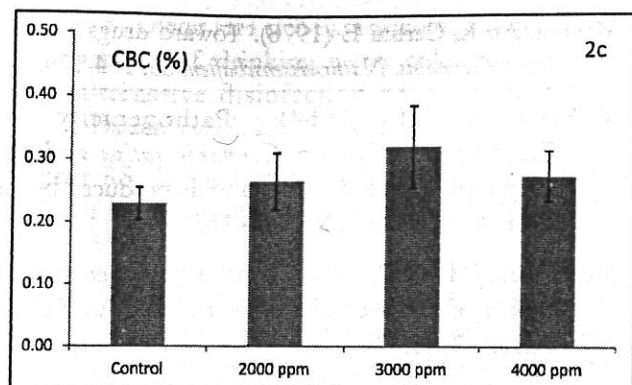


Fig. 2 (a-e). The effect of different concentrations (2000, 3000 and 4000 ppm) of 'stabilized ClO<sub>2</sub> (sodium chlorite) on cannabinoids concentration (a: THC, b: THCV, c: CBC, d: CBG and e: CBN) of cannabis plants. Bar represents mean ± SD (n=6).

#### ACKNOWLEDGMENTS

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