A Study of Antioxidant Activities and GC-MS Analysis of Sodium nitroprusside (SNP) on Zea mays Leaves

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ABSTRACT

The present study was conducted to investigate the foliar applied Sodium nitroprusside (SNP) on antioxidant and bioactive compounds in Zea mays. The plants where treated with different concentration of SNP (5µM and 1mM) on 20 days old seedling Zea mays. The treatment was carried out for 2 days. In general, the 5µM and 1mM increase in the production of proline content, superoxide dismutase (SOD), catalase (CAT), peroxidase (PD) and Malondialdehyde content when compare to the control. Where as in case of bioactive compounds production the maximum significant of bioactive compounds are present in 5µM of sodium nitroprusside. The major constituents were Octasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15–hexadecamethyl (RT: 15.873); 2-Ethylacridine (RT: 16.582); Silicic acid, diethyl bis (trimethylsilyl)ester (RT: 17.395) along with other minor constituents were also present. Hence it is concluded that regular application of SNP to Zea mays resulted in higher biomass production and phytochemical content.

Keywords : Antioxidant, Bioactive compounds, GC-MS, Sodium nitroprusside (SNP) and *Zea mays*

I. INTRODUCTION

Plants have been valuable source of natural products for maintaining human health, and for natural therapies since time immemorial. The popularity of using plants for therapeutic purpose has been identified on the onset where traditional health care using traditional medicine is being provided (WHO, 2010). Antioxidants are usually aromatic compounds, capable of terminating chain reactions via protonation (Torre *et al.*, 2017). Antioxidants radicalize themselves to stabilize free radicals. These then stabilize the charge by delocalization of an electron in their aromatic ring (Flora *et al.*, 2009). An array of secondary metabolites like phenolics(Ayoub *et al.*, 2016), flavonoids (Pietta, 2000), carotenoids (Fiedor and Burda 2014), steroids(Mooradian, 1993) and thiol compounds (Gungor *et al.*, 2011) act as antioxidants. Antioxidants have found a promising place in food industry (Finley *et al.*, 2011) , cosmetics, antiaging products (Masaki, 2010), healthcare (Blomhoff, 2006) and pharmaceutical industry(Brewer, 2011) . Diverse uses of antioxidants and the growing market have given the impetus to discover and develop more effective and safe antioxidants from natural sources. Plants due to their vast metabolic diversity offer great dimensions for exploring new antioxidant compounds. Sodium nitroprusside (SNP) represents one of the most commonly used nitric oxide (NO) donors. It was classified as a phytohormone that might function as a gaseous endogenous plant growth regulator as well as a non-traditional plant growth regulator. Niric oxide

(NO) has now gained significant place in Plant Science, mainly due to its multifunctional role as bioactive molecule in plant growth and development .They are naturally produced within plants and used to regulate the plant growth and developments. In biological sciences, this acts as a signal molecule in plants responsible for the regulation of the expression of many defense-related enzymes. NO has gained increasing interest as important intermediate and intracellular signaling molecule in plant systems which mediates various physiological, biochemical and developmental processes in plants, including seed dormancy, seed germination, primary lateral root growth, floral transition, flowering, stomatal movement, photosynthesis, mitochondrial functionality, senescence, plant metabolism and cell death as well as stress response (Paraiz Ahmad *et al.*, 2016).

Present study was a comprehensive attempt to assess antioxidant activities of different concentration of Sodium nitroprusside on *Zea mays* leaves and study the antioxidant activities Study also attempted to predict the main antioxidant compounds and phytochemical profile maps of all the concentration of Sodium nitroprusside (5µM and 1mM) used in this study with GC-MS detection technique.

II. MATERIALS AND METHODS

Cultivation of seedlings

Healthy and uniform seeds of *Lablab purpureus* were purchased from Agricultural Research Centre, Kovilpatti. The percentage of seed germination was found to be 80-85%. The seeds were sown in pots containing a mixture of red soil, black soil and sand mixed in the ratio of 2: 2: 1. Soon after emergence of the cotyledons, the seedlings were shifted to daylight conditions. Since the ambient climate was too hot for the seedlings, a 40% cut off mesh filter was used to surround the pots for an initial period of 2-3 days.

Exogenous Application of Sodium Nitroprusside(SNP)

Sodium nitroprusside was obtained from Sigma Chemical Co. (St. Louis, U.S.A). SNP was initially dissolved in water and made up to 5µM, and 1mM containing 0.02% Tween-20 (Polyoxy ethylene sorbitanmonolaurate). Each seedling required about 10ml of spray solution. The foliar spray was given for two days early in the morning and growth analyses were done after 20 days of seedling growth. The seedlings were sprayed with solutions until dropping with an atomic sprayer. Plants sprayed with 0.02% Tween-20 served as the control.

After two days of the treatment the seedlings of *Zea mays* were used for measuring the growth parameters The biochemical and enzymatic characters were analyzed by the following methods: *in vivo* nitrate reductase activity (Jaworski, 1971), Polyphenol oxidiase activity (Mukherjee *et al.*, 1975), Superoxide dismutase activity (SOD) Bowler *et al.* (1992) and Malondialdehyde content Heath and pacher (1968).

GCMS Analysis

Plant material and sample preparation

Fresh leaves of *Zea mays* were harvested from the various treatments. Exactly I g of leaves were ground in 5ml of Corbinol-grade in a mortar. The homogenate was filtered through 2 layers of cheese cloth and the filterate was spun at 5000 rpm for 15 minutes. The extracts were then filtered through Whatmann filter paper No. 41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used. 1 µL of these solutions was employed for GC/MS analysis

GC-MS Analysis

GC-MS analysis of these extracts were performed using a Agilent 7820A GC and Agilent 5977E Series

GC/MSD system. The Agilent 7820A GC is ideal for small- and medium-sized labs that are primarily concerned with routine analyses using standard GC methods. Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and Front Injector: Syringe Size-10 Ml, InjectionVolume-1 µL, Injection Dispense Speed - 6000 µL/min, Injection Type –Standard, Injector temperature 280°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da.

III. RESULTS AND DISCUSSION

In the present study, activities of antioxidant enzyme POD increased and followed by exogenous application of NO *via* different modes in *Zea mays.* NO (5µM and 1mM) induced adverse effects on the growth of *Zea mays* by improving the activites of POD and raised the levels of ascorbate, nitrite reductase under saline condition . The findings are is conformation with the earlier reports of Mania *et al.,* (2014). As such, this experiment suggested SNP could partially alleviate the toxic effect. However, the high concentration of SNP could make SOD increased. The content of MDA is an indicator of lipid peroxidation and oxidative damage to the membrane. Therefore, we deduced that the protective role of SNP might be related to its effects on the elimination of MDA. In *Zea mays* 1 mM concentration of SNP increased MDA content. However high concentration of SNP could make catalase activity decrease, and MDA content increase. This is in confirmation with earlier findings of (Aslihan *et al.,* 2015) in Lettuce seedlings.

Gas Chromatography Mass Spectrum (GCMS) Analysis

The extraction of selected plant leaves are play an important role on the herbal drug Formulations. Hence the present study was aimed to find out the bioactive compounds present in the exogenous application sodium nitroprusside of *Zea mays* by using Gas chromatography and Mass spectroscopy. The active compounds with their peak number, concentration (peak area %), and retention time (RT) are presented in Table 1to 2 and Fig. 1 to 2 which shows the presence of 14 bioactive phytochemical compounds on the 5µM exogenous application of sodium nitroprusside of *Zea mays*. Where as in case of 1mM of sodium nitroprusside reduces the presence of bioactive compounds when compare to the control plant. The maximum significant of bioactive compounds are present in 5µM of sodium nitroprusside. The major constituents were Octasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15–hexadecamethyl (RT: 15.873); 2-Ethylacridine (RT: 16.582); Silicic acid, diethyl bis (trimethylsilyl)ester (RT: 17.395) along with other minor constituents were also present (Table 1to 2). The above mentioned isolated bioactive phytochemical compounds posses the many biological activity and further study of these phytoconstituents may prove the medicinal importance in future.

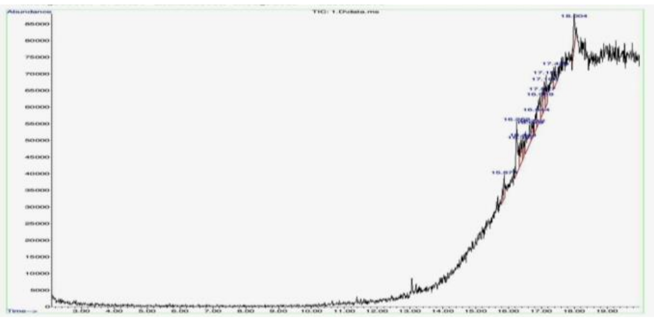

Table. 1. Effect of Control plants (*Zea mays*) on phytochemical compound analysis by GCMS

S.No	Rt (min.)	Compounds	% Area
1	15.873	Heptosiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	6.33
2	16.383	Anthranilic acid, N-methyl -, butyl ester	15.57
3	16.440	Octasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	7.96
4	16.648	Octasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15–hexadecamethyl	4.18
5	16.714	2,4,6 – Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	11.51
6	16.251	N-Methyl- 1-adamantaneacetamide	3.13
7	16.847	Indole-2-one,2,3-dihydro –N-hydroxy-4-methoxy-3.3-dimethyl-	5.19
8	16.960	5-Methyl-2-trimethylsilyloxy-acetphone	10.93
9	17.026	Indole-2-one,2,3-dihydro –N-hydroxy-4-methoxy-3.3-dimethyl-	5.39
10	17.102	2-Ethylacridine	8.85
11	17.149	1-Methyl-3-Phenylinidole	5.64
12	17.423	2-Ethylacridine	6.34
13	18.000	2-Ethylacridine	8.98

Table. 2. Effect of Sodium nitroprusside (5µM and 1mM) foliar spray on phytochemical compound analysis by GCMS of *Zea mays*

S.No	Rt (min.)	Compounds	% Area
1	15.873	Heptosiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	6.33
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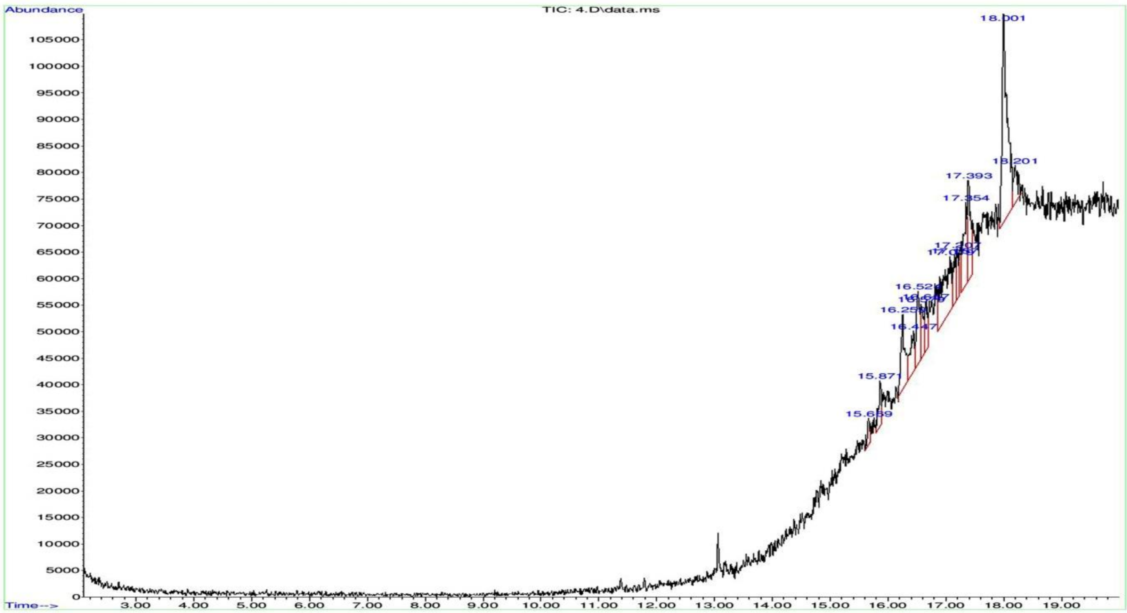
6	16.251	N-Methyl- 1-adamantaneacetamide	3.13
7	16.847	Indole-2-one,2,3-dihydro –N-hydroxy-4-methoxy-3.3-dimethyl-	5.19
8	16.960	5-Methyl-2-trimethylsilyloxy-acetphone	10.93
9	17.026	Indole-2-one,2,3-dihydro –N-hydroxy-4-methoxy-3.3-dimethyl-	5.39
10	17.102	2-Ethylacridine	8.85
11	17.149	1-Methyl-3-Phenylinidole	5.64
12	17.423	2-Ethylacridine	6.34
13	18.000	2-Ethylacridine	8.98



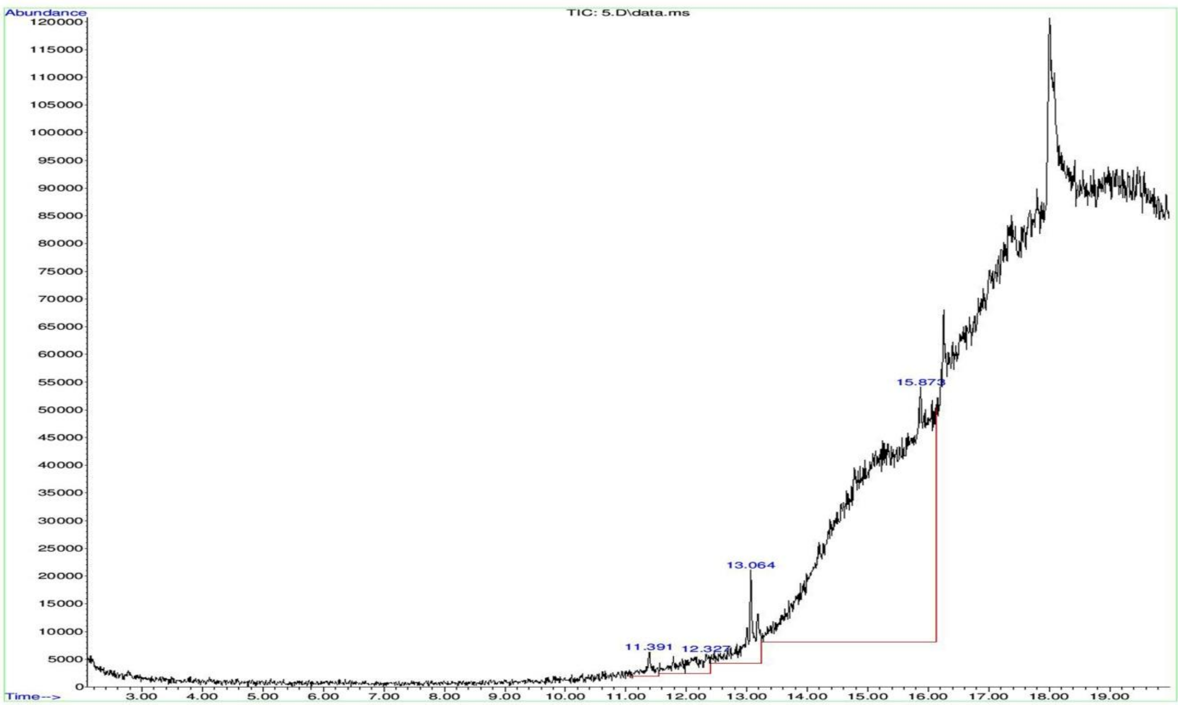


Fig 2. Effect of Sodium nitroprusside (5μM and 1mM) foliar spray on phytochemical compound analysis by GCMS of *Zea mays*

IV. CONCLUSION

The low concentration of SNP has a good potential in improving accumulation of bioactive compounds. The concentrations of 5μm give the best results. So, the farmers may be advised to make up of SNP for improving biomass production of *Zea mays.*

V. Acknowledgement

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VI. REFERENCES

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