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Effect of Simulated Acid Rain on the Carotenoid Content of *Capsicum frutescens* Linn.

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Carotenoids are pro-vitamins as they yield vitamin-A in biological systems. They are anti-oxidants, hence , are important from the point of view of health. Therefore, effect of change in the environment around them due to acid rain was studied in *Capsicum frutescens* Linn. under simulated conditions as they are source of carotenoids. Effect of simulated acid rain of pH 3.0, 4.0 and 5.0 was studied on the carotenoid concentration of leaves of *Capsicum frutescens* Linn. For the determination of concentration UV- visible spectrophotometer was used.

Analysis of data revealed that increased acidity of rain decreased carotenoid concentration in leaves in general due to leaching of mineral nutrients or decomposition of pigment. Carotenoid concentration in leaves showed an increase with number of days at all the three pH which was checked at 45, 60, 75, 90 and 105th days. Out of all the observations made carotenoid concentrations were minimum at 75th days and lowest value was recorded at 75th day of the set sprayed by pH 4.0 acid rain. It may be concluded that *Capsicum frutescens* Linn. Is more prone to damage of carotenoids at pH 4.0 around 75 days.

Keywords: *Capsicum Frutescens* Linn., Simulated Acid Rain And Carotenoids.

Introduction

Acid rain is the rain with pH lesser than 7.0. It contains nitric acid and sulphuric acid. Acid rain has bad effects on herbs, shrubs and trees. It affects directly as well as indirectly to the organisms in contact of which it comes. Oxides of nitrogen and sulphur are released in atmosphere through industries as well as vehicles and remain there. They have chances to get converted in respective acids [Bunce N.J., 1991] [1]. Oxide of sulphur (SO_2) can stays in the form of sulphate ion [SO_4^{2-}] in the atmosphere for three weeks. Acid rain is a mixture of dry and wet precipitation from atmosphere that contains more than normal amount of HNO_3 and H_2SO_4. The sources of acid rain may be natural as well as created by human beings. For instance, decaying vegetation, oxides of nitrogen and sulphur from volcanoes besides industries [Kaur H., 2012] [2]. Acid rain is responsible for geochemical shift in soil water and soil leading to leaching of nutrients. Leaching effects negatively to absorption of nutrients by roots which slows down growth of the plants. Excess of rain water in soil dissolves soil substrates causing soil-erosin. It may also lead to excess of metals to the toxic extent. This phenomena affects differently to plants depending upon its degree of tolerance to environmental changes. As acid rain is formed by the dissolution of NO_2 and SO_2 in rain water, therefore, it contains H^+ ions due to dissociation of acids.

$$CO_2 + H_2O = HCO_3^- + H^+$$
$$HCO_3^- = CO3- + H^+$$
$$2\,SO_2 + CO_2 = 2SO_3 + C$$
$$SO_3 + H_2O = SO_4^{2-} + 2\,H^+$$
$$2\,NO + O_2 = 2\,NO_2$$
$$3\,NO_2 + H_2O = 2\,NO_3^- + NO + 2\,H^+$$

Review of Literature

pH of acid rain increases due to carbon dioxide which produces carbonic acid which is a weak acid and is responsible for about 5.6 pH. 5.6 is the pH of normal rainfall. But, dissolution of **NO_2 and SO_3** lead to lower pH as low as 4.5. Notable effects of acid rain on the crops are in the form of injuries, leaching of nutrients from leaves and lowering of chlorophyll contents have also been observed. Effects on proteins, carbohydrates, yields and nitrogen fixation also occur. Besides, visual injury, yellowing and tissue de-pigmentation i.e. chlorosis have also been reported. Wet acid deposition also kills algae, bacteria and fishes in rivers and ponds which disrupts ecological balance. Effect of acid rain on the growth of forests has been observed in Europe and Northern America, which also reduced agricultural productivity [Sharma B.K., 2001] [3]. Acid rain affects terrestrial vegetation because of reduction in rate of photosynthesis and increase in vulnerability to diseases and growth [Cohen C.J., 1981] [4]. Acid rain retards the growth of broccoli, spinach, peas, beans, potatoes, carrots and radish etc. Acid rain inhibits nitrogen fixation and hence reduces productivity because of decrease in fertility of soil. Acids cause leaching of magnesium from soil which is metallic component of chlorophyll and is essential component of it [Morrison, 1984] [5]. Acid rain has shown destructive effects on vegetation and its generative structures [Nouchi, 1993; Rinallo and Mori, 1996] [6,7]. Acid rain effects net photosynthesis near optimum temperature and light [Momen et al., 1997; Wallin et al., 1990; Tjoelkar et al., 1993; Wallin et al., 1992] [8-11]. Rain of pH 3.0 decreases net photosynthesis of foliage from 11 to 25% depending upon the temperature [Momen et al,, 1999] [12]. It also affects biochemical composition, morphology and phonology of plants [Fredricken et al., 1995; Fincher, 1992] [13,14]. Rate of photosynthesis and concentrations of chlorophyll a and chlorophyll b were found to be decreased by acid rain in *Pinus armandi* [Yunfeng Shan et al., 1996] [15]. Reduction in photosynthetic pigments chlorophyll a and chlorophyll b upon comparison with control were reported in *Solanum melongena* [Sharma and Sharma, 2011] [16] at pH 3.0, 4.0, and 5.0. Cao et al. (2010) [17] reported no effect on chlorophyll contents at pH 4.0, and 5.0. However, Shaukat and Khan (2008) [18] found significant reduction in chlorophyll a and chlorophyll b at pH 3.0, 4.0 in *Lycopersicon esculentum* mill (tomatoes). The acid rain contents NO_2 and SO_2 provide oxygen radicals which may cause inhibition of photosynthesis [Shan, 1998] [19]. Sharma (1996) [20] reported significant reduction in chlorophylls, carotenoids, proteins, carbohydrates, ascorbic acid and phosphorus by pH 4.5, 3.5 and 2.5 acid rains. Kumaravelu and Ramanujam (1998) [21] also reported decrease in chlorophylls, carotenoids and starch contents by showers of pH 2.5 acid rain in *Vigna radiata* cultivar ADT-1. Sharma M. (2019 & 2020) [22,23] studied interactive effect of simulated acid rain and auxin on carbohydrate content and effect on chlorophyll 'a' as well as chlorophyll 'b' content of *Capsicum frutescens* var. Sweet magic.

Capsicum frutescens Linn. Is a small spreading shrub and is cultivated throughout India. Its main chemical constituents include capsaicin and dihydrocapsaicin. Plant is 3-4 feet in height. Its fruits produce burning sensation, increase in appetite and are useful in loss of consciousness, indigestion, delirium, diarrhoea and chronic ulcers. Leaves are useful for the treatment of headache, pain, sores, bronchitis, night blindness, adrenoid, etc. Juice is antimicrobial. Crude juice is remedial for backache, cough, chest troubles and stomach ache [Joshi S.C., 2000] [24]. *Capsicum frutescens* Linn. is of significant nutritive importance because proteins, carbohydrates and ascorbic acid as well as carotenoids are its important constituents. As its leaves contain chlorophyll and carotenoid pigments, hence, it is useful as anti-oxidant also. Besides, it also contains Vitamin-A i.e. thiamine chloride hydrochloride and few minerals [Sharma M., et al., 2015] [25].

Photosynthetic pigments play key role in the synthesis of food by plants. Besides chlorophylls, they also include carotenoids, flavones and anthocyanins [Brown S.B. et al.,1991; Britton G., 1983 and Costache M.A. et al., 2012] [26-28]. Chlorophyll a as well as chlorophyll b are essential leaf pigments for photosynthesis [Nayek S. et al., 2014] [29]. Concentration of chlorophylls and carotenoids in plants show variation depending upon climatic conditions [Shaikh and Dongara, 2008] [30]. Absorbance of U.V.-visible light property of these pigments make feasible their qualitative and quantitative estimations.

Carotenoids are provitamins because they yield Vitamin-A in biological systems [Finar I.L., 1988] [31]. These are antioxidants and are important from the point of view of good health and longevity. Hence, effect of change in environment around them due to acid rain was studied on *Capsicum frutescens* Linn. under simulated conditions, because *Capsicum frutescens* is a good source of carotenoids. Effect of simulated acid rain of pH 3.0, 4.0 and 5.0 was studied on the carotenoid concentration of leaves of *Capsicum frutescens* Linn. For the determination of concentration UV-visible spectrophotometer was used.

Aim of Study

As *Capsicum frutescens* Linn. is an important plant fruits of which are edible and all the contents are distributed in fruits through leaves, hence, effect of simulated acid rain was studied on carotenoid content in its leaves to find if they get enhanced or decreased through UV- visible spectrophotometer. It is worth mentioning that carotenoids are compounds with anti-oxidant properties, therefore, are important from the point of view of health.

Material and Method

Seeds of *Capsicum frutescens* Linn. were sown in soil and 40 saplings of 10 days each were planted in 40 pots. 10 pots were taken as control and 3 other sets of 10 pots each were prepared for the treatment with acid rain of pH 3.0, 4.0 and 5.0. Beside regular watering to plants set wise treatment was given to plants as detailed in Table-1.

Simulated acid rains of pH 3.0, 4.0 and 5.0 were prepared with the help of electronic pH-meter by adding mixture of conc. H_2SO_4 and HNO_3 in the ratio 7:3 (v/v) in distilled water [Lee J.J., 1981] [32].

For the study of carotenoid contents in leaves of *Capsicum frutescens* Linn. Harborne J.B. (1973) [33] protocol was followed. 100 mg of fresh plant leaves were grinded with 10 ml of 80% acetone in a pestle and mortar. Suspension was centrifuged at 3000 rpm for 10 minutes. Supernatant was taken in a test tube and pellet was discarded. Supernatant was made up to 10 ml and readings of O.D. values at 480 nm were recorded by filling half of the cuvette and inserting it in Schimadzu U.V.-visible spectrophotometer. Carotenoid concentrations were calculated according to following formula:

Amount of carotenoid in 100 mg plant tissue

$$= \frac{4 \times \text{O.D. value} \times \text{Total volume of sample (i.e. 10 ml)}}{\text{Weight of fresh plant leaves (i.e. 100 mg)}}$$

$$= \text{---------- mg/ gm of fresh weight}$$

Table -1

Sr. No.	Set No.	Treatment	Periodicity
1.	1st set	Control- water [pH = 6.0]	10 ml sprayed twice a week
2.	2nd set	Acid rain – pH 3.0	10 ml sprayed twice a week
3.	3rd set	Acid rain – pH 4.0	10 ml sprayed twice a week
4.	4th set	Acid rain – pH 5.0	10 ml sprayed twice a week

Results and Discussion

Effect of simulated acid rain of different concentrations i.e. pH = 3.0, 4.0, and 5.0 on carotenoid concentration in the leaves of *Capsicum frutescens* Linn. is shown in Table -2.

Treatment of saplings at pH 3.0 acid rain gave carotenoid contents 81.49 %, 87.70 %, 85.94 %, 76.27 % and 91.28 % of control at the plant ages 45, 60, 75, 90 and 105 days respectively. At pH 4.0 carotenoid contents were 81.85 %, 84.22 %, 63.77 %, 91.68 % and 89.83 % of control at the plant ages 45, 60, 75, 90 and 105 days respectively and at pH 5.0 carotenoid concentrations were 84.57 %, 102.57 %, 83.59 %, 125.19 % and 135.25 % of control at the plant ages 45, 60, 75, 90 and 105 days respectively.

Table -2: Effect of simulated rain (pH 3,0, 4.0 and 5.0) on the carotenoid content (mg/g FW) in the leaves of *Capsicum frutescens* Linn.

S,No	Treatment	45 days	60 days	75 days	90 days	105 days
1.	control	0.2204	0.2180	0.2048	0.2524	0.3580
2.	pH 3.0	0.1796	0.1912	0.1760	0.1925	0.3268
3.	pH 4.0	0.1804	0.1836	0.1304	0.2314	0.3216
4.	pH 5.0	0.1864	0.2236	0.1712	0.3178	0.4872

Carotenoids are present in chromoplasts and provide colours to fruits and vegetables. They are photosynthetic pigments along with chlorophyll [Nayek S., 2014] [29]. According to Vechetal and Ruppel (1992) [34] carotene is photosynthetic pigment which prevents chlorophyll and thylakoid membrane from damage by energy absorbed by peroxidation. Infect, carotenoid is a group of about 70 compounds [Costache M.A. et al., 2012] [28] which absorb sunlight along with chlorophyll. They are important for photosynthetic process which produces food and is responsible for plant growth.

Carotenoid pigments were assessed in the leaves of *Capsicum frutescens* Linn. under the influence of simulated acid rains of pH 3.0, 4.0 and 5.0 during present investigation. It was found that its concentration increases with increase in pH or in other words with decrease in acidity or H^+ ion concentration in acid rain in comparison to control. Lal N. and Singh H.L.(2015) [35] also reported same trend in carotenoid contents in plant *Helianthus annus* L. (sun flower). It was observed by Kausar et

al. (2010) [36] that simulated acid rain lowered concentration of carotenoids at all the pH of acid rain in wheat plant and maximum suppression was recorded at pH 3.0 i.e. at highest H^+ concentration. Peng et al. (2003)[37] found decreased carotenoid concentration in presence of acid rain along with Kumaravelu (1998)[21] in green gram, wheat, maize and rice. The possible explanation for the results during present study is leaching of nutrients or decomposition of pigment under acidic conditions. As revealed by the analysis of data carotenoids in leaves showed increase in concentaration mg/gm of fresh weight with the number of days at all the three pH with increase in number of days from 45 to 105 days. The carotenoid concentration was analysed using U.V.- visible spectrophotometer at 45, 60, 75, 90 and 105 days. Out of all the observations made carotenoid concentrations were minimum at 75th days and lowest value was recorded at 75th day of the set sprayed by pH 4.0 acid rain. It may be concluded that *Capsicum frutescens* Linn. is more prone to damage of carotenoids at pH 4.0 around 75 days which may be due to poor plant strength to compensate the effect of acid rain at early age. Highest carotenoid was at 105th day at pH 5.0 which may be because of better strength at higher plant age and lower acidic strength of acid rain.

Conclusion

It may be concluded from the study that carotenoid content get damaged to certain extent under the influence of acid rain. The trend is that maximum damage is at pH 4.0 and minimum at 5.0. However, carotenoid content is minimum at around 75 days and lowest value was found at 75th day at pH 4.0. It is found that *Capsicum frutescens* Linn. is more prone to damage of carotenoids at pH 4.0 around 75 days which is due to lesser plant strength. Highest carotenoid content at 105th day at pH 5.0 is explainable on account of better resistance at milder acidic strength of acid rain. Decrease in carotenoid content in presence of acid rain may be due to leaching or decomposition of pigments.

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References

1. *Bunce, N.J. (1991, Environmental Chemistry,Wuerz Publishing Limited, Winnpeg, Canada. 146-179.*
2. *Kaur, H. (2012), Environmental Chemistry, Pragati Prakshan, Meerut (India).*
3. *Sharma, B.K. (2001) Environmental Chemistry, Krishna Publication (P) Ltd., Meerut (India). 179-186.*
4. *Cohen, C.J., Grothus, L.C., Perrigan, S.C. (1981). Effect of sulfuric acid rain on crop plants, special report 619, May 1981,Agricultural experimental station, Oregon state University, Corvallis.*
5. *Morrison, I.K., (1984). Acid rain, forests and forestry. Stone, E.L. (Ed.), Proc. 6th North American Forest Soils Conf., University of Tennessee, Knoxville. 209-219.*
6. *Nouchi, I. (1993). Acid precipitation in Japan and its impact on plants to effect of acid precipitation on growth or yield of corps and forest decline. JARQ, 26:231-237.*
7. *Rinallo, C. and Mori, B. (1996). Effects of simulated acid rain on the foliage and fruits yield of Malus domestica Borkh. J. Hort. Sci. 67: 553-559.*
8. *Momen, B., Anderson, P.D. , Helms, J.A. and Haupis, J.L.J.(1997). Acid rain and ozone effects on gas - exchange of Pinus Ponderosa : a comparison between trees and seedlings. Int. J. Plant Sci. 158 : 617-621.*
9. *Wallin, G., Skarby, L. and Sellden, G. (1990). Long term exposure of Norway spruce, Picea abies (L.) Karst. to ozone in open-top chambers. I. Effects on the capacity of net photosynthesis, dark respiration and leaf conductance of shoots of different ages. New Phytol. 115 : 335-344.*
10. *Tjoelker, M.G., Volin, J.C., Oleksyn, j. and Reich, P.B. (1993). Light environment alters response to Ozone stress in seedlings of Acer saccaharum March. And Populus L.I. in situ net photosynthesis, dark respiration and growth. New Phytol. 124 : 627-636.*
11. *Wallin, G., Skarby, L. and Sellden, G. (1992).). Long term exposure of Norway spruce, Picea abies (L.) Karst. to ozone in open-top chambers. III. Effects on the light response of net photosynthesis in shoots of different ages. New Phytol 121 : 387-394.*
12. *Momen, B, Anderson, P.D. and Helms, J.A. (1999). Temperature dependency of acid rain effect on photosynthesis of Pinus ponderosa. Forest Ecol. Manag. 113 : 223-230.*
13. *Fredericken, T.S., Joyce, B.J., Skelly, J.M., Steiner, K.C., Kolb, T.E., Kouterick, K.B., Savage, J.E. and Snyder, K.R.(1995). Physiology, morphology and ozone uptake of leaves of black cherry seedlings, saplings and canopy trees. Environ. Pollut. 89: 273-283.*
14. *Fincher, J. (1992). Comparison of structural changes in red spruce during cold hardening in mature trees and seedlings used in pollution exposure studies. For. Ecol. Manag. 51: 105-113.*
15. *Yunfeng Shan, Zongwei Feng, Takeshi Izuta, Masatoshi Aoki and Tsumugu Totsuka. (1996). Individual and combined effects of ozone and simulated acid rain on growth, gas exchange rate and water use efficiency of Pinus armandi Franch. Environ. Pollut. 91: 355-361.*
16. *Sharma M. and Sharma V.P. (2011), Effect of simulated acid rain on the contents of leaves of Solanum melongena, JPDS, Vol. 3(1&2): 169-173.*
17. *Cao, C.X., Zhou, Q., Han, L.L., Zhang, P., Jiang, H.D. (2010). Effect of simulated acid rain on oilseed rape (Brassica napus) physiological characteristics at flowering stage and yield. Ying Yong Sheng Tai Xue Bao. 21(8): 2057-62.*

18. *Shaukat, S.S and Ali Khan, M (2008). Growth and physiological responses of tomato (Lycopersicon Esculentum Mill) to simulated acid rain. Pak. J. Bot. 40(6):2427-2435.*
19. *Shan, Y. (1998). Effect of simulated acid rain on Pinus densiflora: Inhibition of net photosynthesis by pheophytization of chlorophyll. Water, Air & Soil Pollut.103:121-127.*
20. *Sharma, N.K. (1996). Effect of simulated acid rain on some orchard trees. Ph.D. Thesis, CCS University, Meerut.*
21. *Kumaravelu, G., Ramanujam, M.P. (1998). Impact of simulated acid rain on growth, photosynthetic pigments, cell metabolites and leaf characteristics of green gram. Photosynthetica.35 (1) : 71-78.*
22. *Sharma M. (2019), Interactive effect of simulated acid rain and auxin on carbohydrate content in the leaves of Capsicum frutescens var. sweet magic, Remarking An Analisation, 3(12): E-19 to E-22.*
23. *Sharma M. (2020), Effect of simulated acid rain and auxin on Chlorophyll 'a' and Chlorophyll 'b' content in the leaves of Capsicum frutescens var. sweet magic, Periodic Research, 9(2) : 1-6.*
24. *Joshi S.G. (2000), Medicinal Plants; Oxford and IBH Publishing Co. Ltd. – New Delhi.*
25. *Sharma M., Sharma V.P. and Kumar S. (2015), Effect of auxin and simulated acid rain on sulphur contents in the leaves of Capsicum frutescens var. Sweet magic, JPDS, Vol. 7(6):503-510.*
26. *Brown S.B., Houghton J.D. and Hendry G.A.F. (1991), Chlorophyll breakdown in Scheer H. (Ed): Chlorophylls. Boca Raton, CRC Press, 465-489.*
27. *Britton G. (1983), The Biochemistry of natural pigments; Cambridge University Press, 133-140.*
28. *Costache, M.A., Campeanu, G. and Neata G., (2012), Studies concerning the extraction of chlorophyll and total carotenoids from vegetables, Romanian Biotechnolo. Letters, 17(5): 7702-7708.*
29. *Nayek S,, Choudhary I.H., Jaishree N. and Roy S. (2014), Spectrophotometric analysis of chlorophyll and carotenoids from commonly grown fern species by using various extracting solvents, Res. J. Chem. Sci., 4(9):63-69.*
30. *Shaikh S.D. and Dongara M. (2008), Analysis of photosynthesis pigments in Adiantum lunulatum ferm at different localities of Sindhudurg District (Maharastra), Indian Fern J., 25, 83-86.*
31. *Finar I.L. (1988), Organic Chemistry Vol. 2: Stereochemistry and the chemistry of natural products, ELBS/Longman, Harlow- Essex.*
32. *Lee J.J., Neeley G.E., Perrigan S.C. and Growthaus L.C. (1981), Effect of simulated sulphuric acid rain on yield, growth and foliar injury of several crops, Environmental Experimental Botany, 21: 171-185.*
33. *Harborne J.B. (1973), Phytochemical Methods, Chapman and Hall, London.*
34. *Vechetal B.W. and Ruppel H.G. (1992), Lipid bodies in Eremophaera viridis De Bary (Chlorophyceae), Plant Cell Phys., 31: 41-48.*
35. *Lal N. and Singh H.L. (2015), Effect of simulated acid rain on chlorophyll and carotenoid content of sunflower (Helianthus annuus L.) leaves, Journal of Functional and Environmental Botany, 5(1): 6-10.*
36. *Kauser S., Hussain M.A. and Khan A.A. (2010), Response of simulated acid rain on morphological, biochemical and leaf characteristics of wheat, Trends in Biosciences, Vol.3: 34-36.*
37. *Peng C.X., Peng C., Lin G. and Wen D. (2003), Effects of simulated acid rain on seed germination and seedling growth of crops, Journal of Tropical and Subtropical Botany, Vol. 4: 400-404.*