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Interactive Effect of Auxin and Simulated Acid Rain on the Carbohydrate Content in The Leaves of *Capsicum Frutescens* Var. California Wonder

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Abstract

Carbohydrates are a group of organic compounds consisting of C,H and O usually in a ratio of 1:2:1 and include such well known compounds as sugars, starch, cellulose etc. They are synthesized from CO_2 and H_2O in chlorophyll containing plants during photosynthesis. Their empirical formula is $C_n(H_2O)_n$. (H.S. Srivastava).[1] They are mostly soluble in water and sweet in taste. These are very important for increasing nutritive value of the plant.(V. K. Jain)[2] Therefore studies were carried out to see the effect of simulated acid rain of pH 3.0,4.0and 5.0, Auxin 1x 10^{-5}, 1x 10^{-6} 1x 10^{-7}M concentration and Interactive effect of both Acid rain and Auxin in the following combinations – pH 3.0 +1x10^{-5} ,pH 3.0 +1x10^{-6} ,pH 3.0 +1x10^{-7}M, pH 4.0 +1x10^{-5} ,pH 4.0 +1x10^{-6} ,pH 4.0 +1x10^{-7}M and pH 5.0 +1x10^{-5} ,pH 5.0 +1x10^{-6} ,pH 5.0 +1x10^{-7}M on carbohydrate content in the leaves of *Capsicum frutescens* var. California wonder. Maximum carbohydrate content is seen at 1x 10^{-5} M auxin concentration at 45th day and minimum carbohydrate content is seen at 60th day.

Keywords: Acid rain, auxin. Carbohydrate, *Capsicum frutescens*, California wonder, Interactive.

Introduction

Capsicum frutescens has elliptical and smooth leaves, slender branches. The branches are 30-120 cm. Fruit is a berry. Carbohydrate provides fuel to brain, heart muscles and central nervous system. Fundamentally all organic food stuffs are ultimately derived from the synthesis of carbohydrate through photosynthesis. The major share of energy is provide by the catabolism of carbohydrates. (V.K.Jain)[2] . Capsicum has active ingredient called as capsaicinoids. This is used in the treatment of arthritis, toothache, neuropathic pain, musculoskeletal pain and in microbial infections also.(Rudrapal, M. Et.al.2020)[3]. Beside this carbohydrates stabilize blood cholesterol level. Therefore present study has been done to see that if the Capsicum frutescens can be enriched with carbohydrate by exogenous application of Auxin . If so then nutritive value of Capsicum frutescens can be enhanced and it would be a great help to the society.

Review of Literature

Simona Ioana et. al. (2009)[4] worked on the effects of simulated acid rain on growth and biochemical process in grass (*Lolium perenne*). The study was done to determine the effects of acid rain with pH 3.0 and pH 4.0 on germination and growth of grass. It was noticed that the germination and the growth decreases when the pH of acid rain decreases under stress of simulated acid rain.

Zhou,et.al (2002) [5], worked on the stress effects of simulated acid rain on three woody plants- *Osmanthus fragrana, Chimonanthus praecox* and *Prunus persica*. They investigated the effect of simulated acid rain on chlorophyll content, cell membrane permeability and effect of light and dark conditions on acid rain injury. The results showed that maximum change was noticed in *Osmanthus fragrana* and less change was noticed in *Prunus persica*.

Cao, et.al. (2010)[6] found that when rape plant was treated with weak acid rain (pH 4.0 to 5.0), the growth was stimulated up to some extent in comparison to control. But it had no effect on leaf chlorophyll content, photosynthetic characters, plant biomass and yield. But when acid rain concentration was increased, leaf chlorophyll content, plant biomass, photosynthetic rate decreased gradually. Seed number per pod, pod number per plant, seed weight and seed yield decreased. As the acidity of acid rain increased, the seed number per pod and the pod number per plant decreased significantly while the seed weight was not affected much.

Shaukat and Khan (2008)[8] reported that simulated acid rain treatment of pH 3.0 and 4.0 reduced the chlorophyll 'a' and 'b' content significantly in comparison to control in tomatoes *(Lycopersicon esculentum mill.*). Reduction in pigment synthesis was more in the treatment with simulated acid rain solution of pH 3.0 in comparison to pH 4.0, while at pH 5.0, no significant difference was observed in chlorophyll content. Chlorophyll 'b' was less affected than chlorophyll 'a'.

Ferenbaugh (1976)[8] while working on simulated acid rain on *Phaseolus vulgaris* found that in the chloroplast small starch granules were present. Carbohydrate production was reduced, although the apparent rate of photosynthesis was increased. Below pH 4 simulated acid rain application of Congo red indicator to the acid treated leaf tissues showed the acidification of cell contents.

Chongling et.al. (1995)[9] noticed the effect of simulated acid rain on ecophysiological characteristics of mung bean and maize. It was found that the chlorophyll content, chlorophyll 'a' and 'b' values, leaf viability of mung bean and maize and the pH leaf sap and soil are positively correlated with the pH simulated acid rain

Shan et. al.(1996)[10] found a reduction in net photosynthetic rate per unit chlorophyll a+b content in *Pinus armandii* exposed to acidic rain. The acidic rain contents (mainly SO2 and NOX) generate reactive free oxygen radicles that may cause inhibition of photosynthesis, enzyme breakdown, membrane damage and DNA alterations, all resulting in reduced plant growth. (Hippeli and Elstner, 1996)[11]. Sharma (1996)[12] observed that in some trees like *Carica papaya, Pinus persica, Psidium guajava, Prunus communis* and *Mangifera indica* total carbohydrates, chlorophyll 'a' and 'b', carotenoids, protein content, ascorbic acid and phosphorus content were significantly decreased at pH 4.5, 3.5and 2.5 of simulated acid rain, while sulphur content was increased in all treatments.

Kumaravelu and Ramanujam (1998)[13] showed the impact of simulated acid rain on growth, photosynthetic pigments, cell metabolites and leaf characteristics of green gram (*Vigna radiata* cv. ADT-1 and CO-5). In cultivar ADT-1 after 5 showers at pH 2.5 chlorophyll, carotenoid and starch contents decreased whereas in cultivar CO-5 decrease was noticed after 10 showers of acid rain. In cultivar CO-5 after simulated acid rain treatment the protein was augmented significantly in contrast to total sugar. A seasonal foliar application of pH 3.0 rain had a persistent effect on net photosynthesis and the effect could only be detected when temperature was either below or above of optimal temperature (about 250C) (Momen et.al. 1999)[14].

Fan and Wang (2000)[15] worked on effects of simulated acid rain on chlorophyll content of five hard wood species by exposing them to the treatment of simulated acid rain of pH 2.0, 3.5, 5.0, 6.0 and distilled water (control). Chlorophyll content decreased between simulated acid rain of pH 2.0 and pH 3.5 and minimum amount of chlorophyll was noticed at simulated acid rain of pH 2.0 in all the species. Between pH 5.0 and pH6.0, maximum chlorophyll content was observed.

Raj et. al. (2003)[16] reported significant decrease in chlorophyll content, carbohydrate, protein and nitrogen content due to lowering in pH level of acidic rain in *Triticum aestivum cv. raj*.3077. Devpura and Khan (2003)[16] also found decreased chlorophyll content with decreasing pH levels of acidic rain in *Phaseolus aureus*. Muduroglu et. al. (2005)[17] reported that after 24 and 48 hours implementation of simulated acid rain on strawberry crops, the vitamin A, C and E levels of plants sprayed with simulated acid rain decreased in respect of pH and time when compared with control.

It was reported by Tong and Liang (2005)[18] that spraying simulated acid rain on shoot of wheat seedlings on acidified soils caused a rapid decrease in the soluble sugar and nitrogen contents of wheat seedlings and reduced some of their physiological activities.Intensive acid rain of pH 3.0 had an obvious harm to physiological activity of wheat seedlings. With the increasing acidity of simulated acid rain, the contents of P and soluble protein in eggplant leaves increased significantly, first reaching the highest at pH 4.0 and3.0 and then decreased, while soluble sugar contents were affected adversely (Zhang et. al.2005)[19].

Kumari and Tomar (2009)[20] also worked on effect of acid rain on two cultivars of *Ocimum sanctum* L.(cv.IC-75730 & cv. local). The sample plants were treated with simulated acid rain of pH 4.5, pH 3.5 and pH 2.5. It was found that carbohydrate

content was adversely affected with increasing acidity and plant age. It was also found that the reduction was more in cv. local than in cv.IC-75730.

Simona Ioanna et.al. (2009)[4] studied the effect of simulated acid rain on chlorophyll content. They found that the chlorophyll content was 18.35 mg/gm of leaves compared to control sample where it was 23.076mg/gm of leaves at pH 3 simulated acid rain.

Abu-Grab , et.al .(2000)[21] , determined that auxin (indole-acetic acid) has a significant increase in dry weight per plant, chlorophyll 'a', chl 'b', chl 'a+b', total carbohydrate and seed germination also.

Hanafy Ahmed. et. al. (2012)[22] recorded that trees treated with 100 M IAA conc. show highest sugar concentration. Treatment of auxin and naphthalene acetic acid on bean plants increases sucrose and polysaccharide content. According to Altman and Wareing (1975)[23], enhancement of sugar concentration and IAA treatments are closely related to each other. Treatment of plants with 100 and 200 M IAA concentration also showed significant and non-significant increase in total free amino acid concentration.

Mostafa and Alhamd (2011)[24] found that germination percentage, plant height, number of branches and leaves, total chlorophyll and dry weight significantly increased when *Balanites aegyptiaca* seeds were soaked in different concentrations of IAA as compared with control. They found that Protein content and carbohydrate content also increased with different concentrations of IAA and GA3 when compared with untreated plants. According to them, plant hormones exert far reaching effects on plant growth and it depends on the concentration of the substance present and the sensitivity of the part concerned.

Taslima, K. et.al. (2011)[25] studied the effect of Indole-3 Acetic Acid (IAA) on biochemical responses of Cowpea (*Vigna unguiculata* (L.) Walp) using foliar spray of different concentrations (20, 40, 60 and 80 ppm of IAA) and found that different biochemical parameters reacted differently. It was found that moisture content decreased with IAA treatment at all concentrations. Highest protein content (22.7%) was recorded with 80 ppm treatment. The best stimulation was found with treatment of 60 ppm IAA recording highest carbohydrate content (60.8%) and calorie content from the seeds of plants treated with 60 ppm IAA. The results indicated that right treatment of IAA concentrations can result in better quality of seeds and leaf pigments of cowpea.

Saba Khalid et. al. (2013)[26] worked on effect of indole acetic acid on morphological, biochemical and chemical attributes of two varieties of Maize (*Zea mays* L.) under salt stress. They reported that salinity affects the rate of growth and gaseous exchange parameters of the two varieties of maize adversely. According to them plant hormone plays a great role in plant development .In their development IAA plays a great role by increasing the photosynthetic process and by changing the activities of spreading leaves in plants. IAA starts the movement of carbohydrates during their manufacture. (Awan, I.U., et.al.(1999)[27].

Bamidele J.F.(2015)[31] noticed that increased acidity levels had negative impact on growth and yield in *Capsicum annuum*. Khalid et.al.(2013) [26] found that introduction of maize to toxic levels of IAA and salt stress causes a number of interrelated structural functional procedures in salt stressed plants In *Helianthus annuus* acid rain application showed early leaf abscission, reduction in pollen germination percentage and pollen tube length (Nand lal et.al.,2017)[32] . Sharma M. (2019) [33] noticed that carbohydrate content is maximum at 105th day. At 60th day minima is seen at all the concentrations. At 1x 10-7 M most significant rate is noticed. Pronounced foliar symptoms were observed in SAR treatment at pH 3.0 , while chlorosis, early leaf senescence and necrosis were less pronounced in SAR at pH 5.0, 5.5, 6.0 (Gufran Ahmed et. Al.(2019)[34]. (Sharma, M,2020).[35] found that Chlorophyll-'a' is maximum at 105th day of treatments with pH 4.0+1x10^{-7} M. Change in chlorophyll-'b' content is through V- turn at prominent combination of pH 5.0+1x10^{-5} M; the maximum value is at the two ends (45th and 105th day). The increase in chlorophyll content may be attributed to higher potential of auxin. Higher concentration of auxin enhances chlorophyll content, through ethylene hormone synthesis. However the effect of acid rain and auxin has not been studied on carbohydrate content in the leaves of *Capsicum frutescens* var. California wonder

Objective of the study

Acid rain is considered to adversely affect the crops in general affecting its growth, nutritive value and yield. In this context, study was done to understand the impact of (a) acid rain on the carbohydrate content of Capsicum frutescens of california wonder variety and also (b) can nutritive value of the vegetable be improved by application of Auxin growth hormone.

Material and Methods

Simulated acid rain of different grades of pH (3.0, 4.0, 5.0) is prepared with the help of electronic pH meter by adding mixture of Sulphuric Acid H_2SO_4 and HNO_3 in ratio of 7:3 (v/v) in distilled water (Lee, J.J.,1981)[28].

Simultaneously Auxin (IAA) solution of 10^{-4} Molar is prepared by dissolving 17.5 mg Auxin in 1000 ml of distilled water. Now from this stock solution Auxin solutions of 1x 10^{-5}, 1x10-6, 1x10-7 M concentrations are prepared after diluting as per requirement.

After this treatment of acid rain of different pH and Auxin of different molar concentration is given to the plants in the form of spray alone as well as in combinations as proposed earlier..Now carbohydrate content is tested in the leaf of *Capsicum frutescens* at a regular interval of 15 days starting from 45th day till 105th day.

Estimation of Carbohydrate

Quantification of carbohydrate content is done as per protocol developed by Anthrone method (Hedge et.al., 1962)[29].[H1]

Carbohydrates are first hydrolysed in to simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm.

100 mg oven dried powdered leaves of the control and treated plants are taken. Transferred sample in to eppendorf tube. Now added 1 ml of 2.5 N HCL. Kept eppendorf tubes in stand. Placed it in the boiling water bath for 3 hour. Cooled the mixture to room temperature. Now added a pinch of sodium bicarbonate slowly to it until the colour disappears and no CO2 releases. Now Centrifuged at 13000 rpm for 15 minutes. Taking 10µl of supernatant in a test tube and added 990 µl distilled water in test tube. Added 4 ml of anthrone (ice cold).Vertexed it (mix well). Kept it in water bath for 8 to 10 minutes. Cooled it to room temperature and O.D. at 630 nm with U.V. spectrophotometer is recorded. Carbohydrate content is calculated with the help of calibration curve.

Results and Discussion

Effect of simulated acid rain of different concentrations (pH 3.0, 4.0 and 5.0) on carbohydrate content, in the leaves of *Capsicum frutescens* var. *California wonder* is shown in Table-1. Indicates that the treatment with acid rain (pH 3.0), carbohydrate content is 191.32%, 121.00%, 16.57%, 72.72% and 55.19% and at the treatment with acid rain (pH 4.0), carbohydrate content is 209.94%, 144.00, 85.67%, 89.46% and 75.60%. The carbohydrate content is 145.98%, 127.00%, 85.275, 86.13% and 99.73% of control at the plant age of 45, 60, 75, 90 and 105 days respectively at treatment with acid rain (pH 5.0).

Table-2 shows the effect of treatment of 1x10-5, 1x10-6, 1x10-7 M auxin on carbohydrate content in the leaves of *Capsicum frutescens* var. *California wonder*. When the plants are treated with 1x10-5 M auxin the carbohydrate content is 142.12%, 159.86%, 125.66%, 579.73% and 135.34% of the control. At 1x10-6 M, the carbohydrate content is 238.08%, 128.00%, 91.20%, 97.18% and 70.30 % of control where as at treatment with 1x10-7 M, carbohydrate content is 231.74%, 114.00%, 51.21%, 83.88% and 69.13 % of the control at the plant age of 45, 60, 75, 90 and 105 days respectively.

Table-3 shows the Interactive effect of treatment of simulated acid rain and auxin on carbohydrate content in the leaves of *Capsicum frutescens* var. *California wonder*. It is found that when the plants are treated with acid rain and auxin (pH 3.0+1x10-5 M), the carbohydrate content is 175.52%, 120.33%, 68.99%, 64.54% and 102.04 % of the control , at the treatment of acid rain and auxin (pH 3.0+1x10-6 M), the carbohydrate content is 169.04%, 279.33%, 129.92%, 74.58% and 48.68% and at the treatment of acid rain and auxin (pH 3.0+1x10-7 M), the carbohydrate content is

202.07%, 255.33%, 63.00%, 76.49% and 37.50% of the control at the plant age of 45, 60, 75, 90 and 105 days respectively.

At the treatment of acid rain and auxin (pH 4.0+1x10-5 M), the values carbohydrate content are 182.25%, 60.00%, 89.01%, 78.80% and 68.86% , when the treatment of acid rain and auxin (pH 4.0+1x10-6 M) is given , carbohydrate content is 233.86%, 13.67%, 28.14%, 81.00 % and 48.41% and at the treatment of acid rain and auxin (pH 4.0+1x10-7 M), the carbohydrate content are 180.83%, 146.33%, 73.59%, 82.69% and 50.25% of the control at the plant age of 45, 60, 75, 90 and 105 days respectively.

When the plants are treated with acid rain and auxin (pH 5.0+1x10-5 M), the carbohydrate content are 211.92%, 444.00%, 98.79%, 92.50% and 51.26 %, at the treatment of at the treatment of acid rain and auxin (pH 5.0+1x10-6 M), the carbohydrate content are 166.84%, 160.67%, 94.25%, 74.92% and 56.32% and when the treatment of at the treatment of acid rain and auxin (pH 5.0+1x10-7 M) is given, the carbohydrate content is 192.49%, 31.00%, 102.42%, 81.29% and 31.22% of the control at the plant age of 45, 60, 75, 90 and 105 days respectively.

Carbohydrate content increases up to 60 days at all concentrations. Afterwards it decreases significantly at pH 3.0. pH 4.0and at pH 5.0 the decrease is not significant. During this period the increase in chlorophyll content may be the cause of increased carbohydrate content. The hike in chlorophyll content increases the rate of photosynthesis, which ultimately forms more food, which is stored in the form of carbohydrate.

Further the increased level of acidity leads to decrease in chlorophyll resulting in decrease in carbohydrate content. The work of Ferrenbough (1976), Forshine (1983), Zhang et. al. (2005) and Shaukat and Khan (2008) help validate results found by experiments on carbohydrate contents.

At 1x10-5 M auxin, the value of carbohydrate increases. At 1x10-6 M, the value of carbohydrates increases only on the 45th day showing decrease afterwards. At 1x10-7 M, the value increases on the 45th day, then decreases. Maximum value of carbohydrate is observed at 1x10-5 M on 45th day, while minimum amount is seen at 1x10-7 M on the 75th day. It can be concluded that the optimum concentration of Auxin for the production of carbohydrate content in leaves is 1x10-5 M.

Best combination of acid rain and auxin that favours carbohydrate content is pH 5.0+1x10-6 M, though variable trends of increase with plant age are observed. The minima on concentration graph is visible at pH 4.0+1x10-6 M auxin on 60th day. Therefore, the optimum combination for carbohydrate content is pH 5.0+1x10-6 M. Degrading effect due to acid rain is compensated for by Auxin 1x10-6 M auxin concentration for production of carbohydrate.

Increase in number of chloroplast increase the concentration of carbohydrate. (Wager et. al., 1954)[30]. Work by Altman and Wereing (1975)[23] supports that IAA treatment given to the plants increases the sugar concentration.

Conclusion

Most significant results are at 1x 10-5 M concentration at which maximum carbohydrate content is observed. Best combination is pH 5.0 + 1x 10-6 M. Most important day is 45th day. Minimum concentration of carbohydrate content is observed at 60th day.

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Table 1 : Effect of simulated acid rain (pH 3.0, 4.0, 5.0) on Carbohydrate content (mg/100 mg ±SD dry wt.) in the leaves of *Capsicum frutescens* var. *california wonder*

Treatment	PLANT AGE (in Days)				
	45	60	75	90	105
Control	0.36 ±0.09	0.14 ±0.04	0.81 ±0.08	0.82 ±0.04	2.38 ±0.16
3.0	0.68* ±0.17	0.17 ±0.03	0.13** ±0.04	0.60* ±0.12	1.31** ±0.28
4.0	0.75* ± 0.13	0.20 ± 0.04	0.69 ± 0.07	0.74* ± 0.03	1.80** ± 0.10
5.0	0.52* ± 0.04	0.18 ± 0.04	0.69 ± 0.02	0.71 ± 0.14	2.38 ± 0.14
NB **=.01 level of significance *=.05 level of significance					

Table 2 :Effect of Auxin (1x10-5,1x10-6,1x10-7 M) on Carbohydrate content (mg/100 mg dry wt., ±SD) in the leaves of *Capsicum frutescens* var. *california wonder*

Treatment	PLANT AGE (in Days)				
	45	60	75	90	105
Control	0.36	0.14	0.81	0.82	2.38

	±0.09	±0.04	±0.08	±0.04	±0.16
1X10^{-5} M	5.06** ± 0.92	2.21** ± 0.51	1.01* ± 0.10	4.76** ± 0.92	3.22** ± 0.17
1X10^{-6} M	0.85** ± 0.04	0.18 ± 0.03	0.73 ± 0.10	0.80 ± 0.08	1.67** ± 0.16
1X10^{-7} M	0.83** ± 0.04	0.16 ± 0.03	0.41** ± 0.04	0.69** ± 0.02	1.65** ± 0.10
NB **=.01 level of significance *=.05 level of significance					

Table 3 : Interactive Effect of simulated acid rain (pH 3.0, 4.0, 5.0)and Auxin (1x10-5 ,1x 10^{-6}, 1x10^{-7}M) on Carbohydrate content(mg/100 mg ±SD dry wt.) in the leaves of *Capsicum frutescens* var. *california wonder*.

Treatment	PLANT AGE (in Days)				
	45	60	75	90	105
Control	0.36 ±0.09	0.14 ±0.04	0.81 ±0.08	0.82 ±0.04	2.38 ±0.16
3.0 +1X10^{-5} M	0.63* ± 0.13	0.17 ± 0.03	0.56* ± 0.07	0.53** ± 0.06	2.43 ± 0.01
3.0 + 1x10^{-}6 M	0.60* ± 0.10	0.39** ± 0.05	1.05** ± 0.03	0.61** ± 0.03	1.16** ± 0.12
3.0 +1x10-7 M	0.72** ± 0.06	0.35** ± 0.03	0.51** ± 0.03	0.63** ± 0.02	0.89** ± 0.11
4.0 +1X10-5 M	0.65** ± 0.06	0.08 ± 0.04	0.72 ± 0.03	0.65* ± 0.06	1.64** ± 0.19
4.0 + 1x10^{-}6 M	0.83** ± 0.10	0.02* ± 0.03	0.23** ± 0.06	0.67** ± 0.04	1.15** ± 0.11
4.0 +1x10-7 M	0.65** ± 0.06	0.20 ± 0.03	0.59* ± 0.07	0.68** ± 0.03	1.20** ± 0.11
5.0 +1X10-5 M	0.76** ± 0.06	0.62** ± 0.04	0.79 ± 0.05	0.76 ± 0.05	1.22** ± 0.08
5.0 +1x10^{-}6 M	0.60* ± 0.08	0.22 ± 0.04	0.76 ± 0.05	0.62** ± 0.05	1.34** ± 0.07
5.0 +1 x10-7 M	0.69** ± 0.05	0.04* ± 0.03	0.82 ± 0.05	0.67** ± 0.04	0.74** ± 0.07
N.B. **=.01 level of significance *=.05 level of significance					