Review Article

Physiological and Biotechnological Approaches in Enhancing Quality Parameters of Chilli

ABSTRACT

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- Physiological approaches play a crucial role in optimizing the plant growth, yield and quality parameters in chilli cultivation. Some key physiological approaches and their influence on quality parameters are stress induction (water and salt stress), hormonal treatment and nutrient management (organic and inorganic fertilizers). These physiological approaches helps chilli growers to meet market demands and consumer preferences more effectively. Biotechnological approachesalso can bring major improvements in the quality aspects of chilly. Also the optimization of yield and quality contributes to the economic success of chili cultivation, providing consumers with high-quality products and producers with sustainable production systems.

Keywords: Physiologicaland Biotechnological approaches, Chilli,Stress, Hormones, Nutrient

1. INTRODUCTION

Physiological approaches in chili cultivation focus on understanding and manipulating the biological processes of plants so asto enhance the growth, yield and quality parameters, such as pungency, colour and nutritional content. Physiological approaches, when implemented effectively, can significantly influence the quality parameters of chili peppers, allowing the growers to meet market demands and consumer preferences. By understanding and managing the physiological responses of plants, producers can optimize both yield and quality, contributing to the economic success of chili cultivation.Biotechnological approaches also play significant role in improving the quality aspects of chilly. Here are some key physiological and biotechnological approaches and their influence on the quality parameters of chili peppers:

2. STRESS INDUCTION

Abiotic Stress and Biotic stresses: Introducing mild stress conditions, such as salinity or water deficit, can stimulate secondary metabolite production, enhancing pungency and antioxidant properties.

2.1 Water Stress

Water deficit creates a stress response in plants, leading to the activation of various metabolic pathways, including those responsible for secondary metabolite production. Under drought condition, chili peppers often show increased levels of capsaicinoids, resulting in higher pungency. Also stress conditions can induce the accumulation of antioxidant compounds and enhance the ability of plants to scavenge free radicals.

Ruiz-Lau *et al*. (2011) investigated the effect of water stress on plant development, capsaicinoid accumulation and capsaicin synthase activity. Water stress was found to reduce plant growth in terms of biomass accumulation and fruit size. The study also showed a decrease in leaf area and plant height under water-deficit conditions, indicating that water stress can inhibit overall vegetative growth. Despite the reduction in growth, water-stressed plants exhibited an increase in capsaicinoid concentrations in the fruit, particularly capsaicin and dihydrocapsaicin. The increase in capsaicinoid content indicated that under water stress, the plant allocates more resources towards secondary metabolite production as a defense mechanism. Environmental factors including water and nutritional stress also may have an impact on the accumulation of capsaicinoids in the fruits and the effect may differ even amongst genotypes within the same species. The habanero pepper (*Capsicum chinense* Jacq.) grown in the Yucatán is highly specific because of its distinct flavor, scent, and pungency. Concentrations of capsaicin and dihydrocapsaicin in the pericarp and seeds of chilli were examined and when compared to control plants, the fruits of stressed plants showed a rise in capsaicin and dihydrocapsaicinat 25 and 45 days after anthesis. On the other hand, the capsaicin synthase activitydecreased in response to water stress and this impact wasfound dependent on the age of plantsand the intensity of the stress. The activity of enzymes may also be regulated by factors other than those involved in their absolute expression levels, such as post-translational modifications or substrate availability. Capsaicin synthase activity produces capsaicin via condensation of the acid 8-methyl-6-nonen (fatty acid pathway) and vanillylamine(phenylpropanoid pathway). The capsaicin synthase enzyme is selectively localized in the placenta, as revealed by immunolocalization studies. The placentais the principal site of capsaicinoid synthesis and accumulation. The placenta of fruits is the primary part responsible for the accumulation of capsaicinoids. The amount of capsaicin synthase activity in Habanero peppers as influenced by water stresswas studied by Narasimha *et al*. (2006).Capsaicinoids break down in part due to peroxidasesand the peroxidase enzymes are involved in the degradation of capsaicinoids and a decrease in capsaicinoid levels was found in fruits. Also in*C. chinense* fruits, capsaicinoid accumulation peaked between 45 and 50 days after anthesis(DAA), while maximal peroxidase activity was found at 60 DAAand at this period capsaicinoidswas found to drastically decrease Ruiz-Lau *et al*. (2011). This study has also shown how the fruits of hot chili pepper regulate capsaicinoid metabolism in response to abiotic stress. However the results indicate that moderate water stress can be used strategically to enhance the pungency of Habanero peppers by increasing capsaicinoid content without significantly harming plant health. Different genotypes may respond differently to water stress and so understanding the specific responses of various Habanero varieties can help breeding programs aimed at developing stress-tolerant cultivars with high pungency. Understanding the relationship between fruit age, enzyme activity, and capsaicinoid levels can help determining the optimal harvest time to maximize pungency and quality (Ruiz-Lau *et al*., 2011).Every seven (T1) or nine (T2) daysinterval one litre of water supplied and plants provided with daily water(T3) which was taken as contro (C).Both water stress treatments led to a significant increase in the concentration of capsaicin content and it was about 16 mg g^{-1} dry weight (DW) with respect to control and andalso it also increased dihydrocapsaicin about 19 mg g^{-1} DW with respect to controland both were observed in the placental tissuesof fruits at 45 DAA.

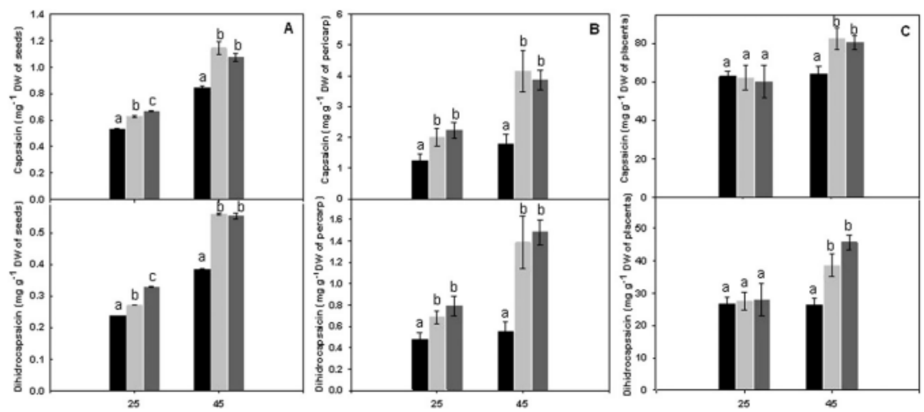


Figure 1. Effect of water stress on capsaicin and dihydrocapsaicin contents in (A) seeds, (B) pericarp and (C) placentas in (--) Daily watering (control), (--) watering each 7 d (T1), (--) watering each 9 d (T2).at both 25 days and 45 (DAA)

Similarly (Jeeatid et al., 2017)studied on the effect of water stress on four cultivars of hot pepper (C. chinense) named AkaneePirote, Bhut Jolokia, Orange Habanero, and BGH1719' . These were applied with different watering schedules: daily irrigation (control; S1), every two days (S2), every three days (S3), and every four days (S4).The amount of capsaicinoidsvaried depending on the cultivar and the degree of stress. Under the S2 treatment, AkaneePirote resulted in considerably greater capsaicinoid content (6,71 SHU) than the control plants (4,69 SHU) it mainly attributed from an increased absolute capsaicinoid content and reduced dry fruit yield.But the capsaicinoid content of other cultivars receiving the S2 treatment did not change much from the control.

2.2 Salinity Stress:

Salinity stress affects ion balance and osmotic pressure within plant cells, triggering defense responses that include the production of secondary metabolites. Similar to water deficit, salinity stress can lead to increased synthesis of capsaicinoids as part of defensive strategy of plants. Salt stress often results in elevated levels of phenolic compounds, which contribute to the antioxidant capacity of peppers.

Four genotypes of Capsicum annuum L. (Caro F1, Berenyi F1, Somorka, and Novosadka) when subjected to two different levels of salt stress(20 mM and 40 mM NaCl stress)the pericarp of the cultivar "Novosadka" exhibited higher total capsaicinoid concentration (17.5% and 50% respectively) compared to the control.Also the total phenol content in the "Somborka" pericarp showed an increaseof 708.80 mg 100 g -1 DW in the 20 mM NaCl treatment and 1161.06 mg 100 g -1 DW in the 40 mM NaCl treatment,revealing that salt had a detrimental influence on the phenol level in the placenta of "Somborka"genotype (Zamljen et al., 2022).

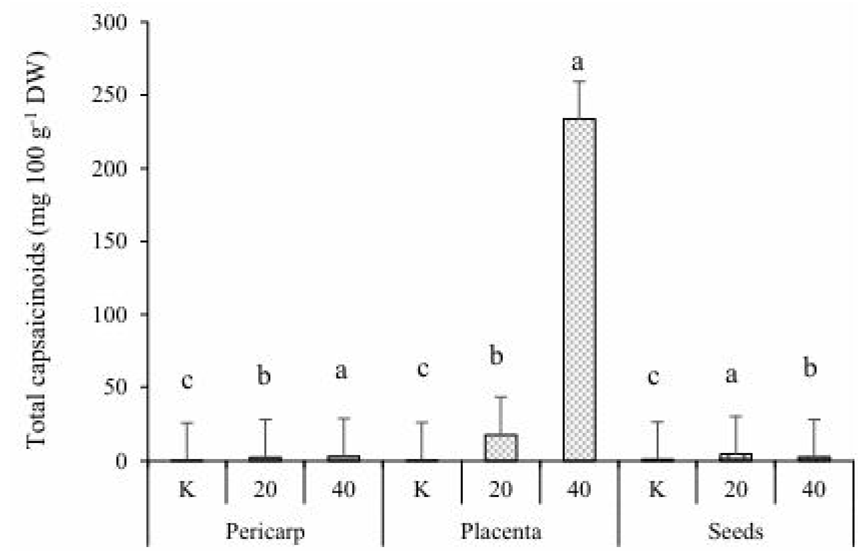


Figure 2. Effect of salt stressed pepper on capsaicin content of genotype ‘Somborka’, divided into three fruit parts. a, b, c indicates statistical differences among the NaCl treatments and control. K = control; 20 = 20 mM NaCl; 40 = 40 mM NaCl.

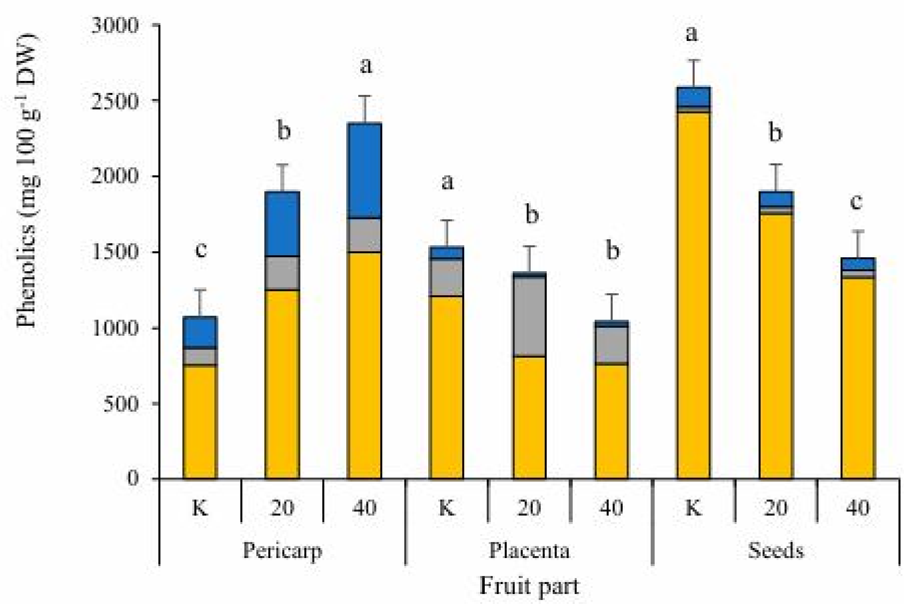
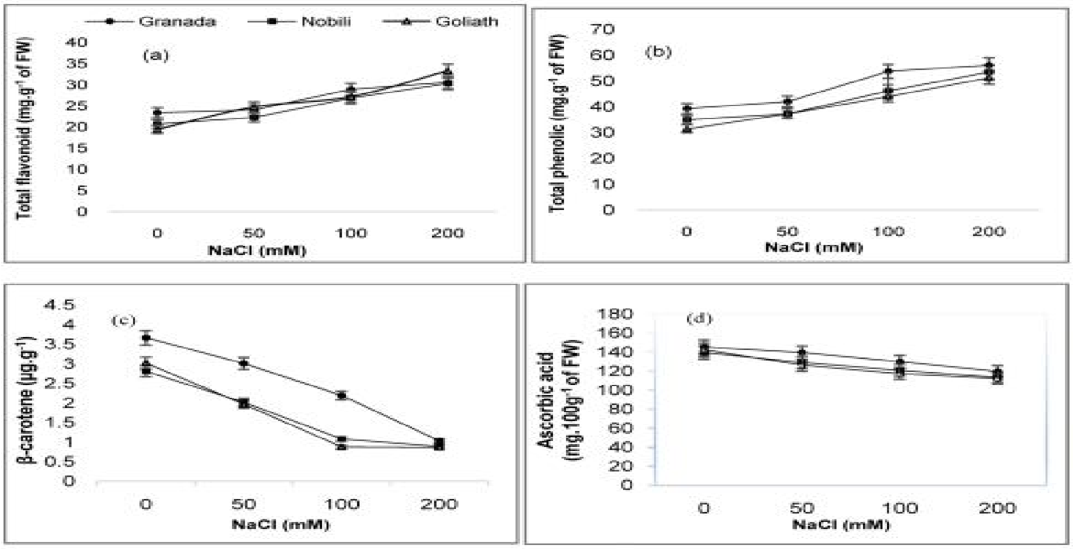


Figure 3. Effect of salt stressed pepper on total phenolics contents of genotype ‘Somborka’, a, b, c indicates statistical differences among the NaCl treatments and control. K = control; 20 = 20 mM NaCl; 40 = 40 mM NaCl.

Hand *et al*. (2021) examined the effects of four different concentrations of NaCl (0, 50, 100, and 200 mM) on the ascorbic acid, antioxidant, and organic and inorganic components (Cu, P, Mn, S, Fe, K, Zn, Ca and Mg) in the fruits of three different pepper fruit cultivars (Granada, Goliath, and Nobili) at the maturity stage. Salinity was found to reduce the mineral content, relative water content, and agro-morphological parameters of pepper fruits. Salt stress was found to affect the concentration of soluble sugars and organic acids also. Moderate salinity (50 and 100 mM) often increase the accumulation of compounds, which play role in osmotic adjustment and stress tolerance. Each cultivar showed different patterns in the accumulation of organic compounds, reflecting their specific metabolic responses to salinity. Notable increases in Na, soluble proteins, proline content, fructose, glucose, and antioxidants such as flavonoids and total phenolics were also observed in salinity. Depending on the genotype, salinity led to increased levels of Na+ and Cl- ions in the fruits, while the concentration of essential nutrients such as K+, Ca2+, and Mg2+ in the fruits varied depending on the cultivar and salt level. High salinity (200 mM) resulted in nutrient imbalance, which affectedthe fruit quality and plant health. Ascorbic acid (vitamin C) content was found to increase with moderate levels of salt stress (50 and 100 mM NaCl) in all the three cultivars. Ascorbic acid is a major antioxidant that helps protecting cells from oxidative damage. At the highest salinity level (200 mM NaCl), ascorbic acid content decreased, indicating that excessive salt stress may overwhelm the ability of plants to maintain higher level of this antioxidant. "Granada" was found to be more resilient and robust in terms of physiological, biochemical, and agro-morphological characteristics than the other types, indicating that it can be cultivated in soils impacted by salinity.



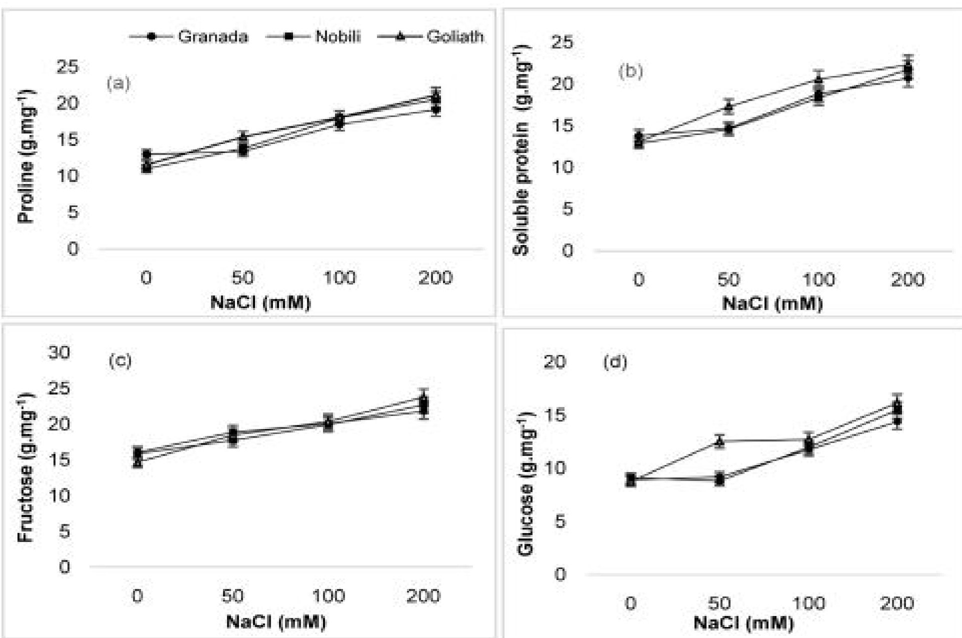


Figure 4. Effect of salt on accumulation of organic compounds in pepper cultivars

Cultivar	Treatment (mM NaCl)	Na	K	Ca	Mg	P	S
Granada	0	4.88 ± 0.01^{bc}	263.71 ± 2.43^{a}	12.89 ± 0.21^{a}	16.81 ± 0.34^{a}	36.81 ± 0.39^{a}	12.39 ± 0.31^{a}
	50	5.37 ± 0.04^{b}	257.57 ± 2.47^{b}	13.97 ± 0.23^{a}	15.12 ± 0.37^{a}	32.34 ± 0.41^{b}	10.86 ± 0.29^{a}
	100	8.14 ± 0.07^{a}	222.72 ± 2.49^{e}	11.39 ± 0.19^{a}	13.99 ± 0.24^{ab}	29.33 ± 0.44^{bc}	11.53 ± 0.25^{a}
	200	10.62 ± 0.08^{a}	201.26 ± 1.83^{f}	11.77 ± 0.25^{a}	11.30 ± 0.42^{b}	28.59 ± 0.42^{c}	8.63 ± 0.23^{b}
Nobili	0	5.39 ± 0.03^{b}	243.19 ± 2.39^{d}	13.27 ± 0125^{a}	15.57 ± 0.39^{a}	33.53 ± 0.38^{ab}	11.69 ± 0.19^{a}
	50	6.46 ± 0.05^{b}	221.47 ± 2.42^{e}	10.82 ± 0.26^{ab}	16.19 ± 0.36^{a}	34.80 ± 0.36^{a}	9.31 ± 0.18^{ab}
	100	8.80 ± 0.06^{a}	205.79 ± 3.44^{f}	11.43 ± 0.19^{a}	12.82 ± 0.41^{b}	31.09 ± 0.32^{b}	7.62 ± 0.21^{b}
	200	10.88 ± 0.09^{a}	190.15 ± 2.51^{g}	9.93 ± 0.22^{c}	10.91 ± 0.39^{bc}	27.15 ± 0.38^{c}	8.35 ± 0.18^{b}
Goliath	0	5.15 ± 0.10^{b}	251.81 ± 2.42^{c}	12.81 ± 0.19^{a}	14.94 ± 0.37^{a}	30.26 ± 0.42^{bc}	11.90 ± 0.23^{a}
	50	7.41 ± 0.08^{ab}	218.53 ± 1.44^{e}	10.26 ± 0.21^{a}	11.92 ± 0.29^{b}	31.42 ± 0.44^{b}	8.92 ± 0.22^{b}
	100	9.64 ± 0.07^{a}	192.45 ± 2.47^{g}	8.88 ± 0.26^{c}	10.28 ± 0.24^{bc}	23.77 ± 0.41^{d}	7.41 ± 0.21^{b}
	200	11.22 ± 0.09^{a}	187.68 ± 1.54^{h}	8.27 ± 0.23^{c}	8.12 ± 0.28^{c}	18.20 ± 0.39^{e}	6.67 ± 0.19^{bc}

Table 1. Effect of salt stress on ions concentrations (mg in 100 g of fresh weight) of pepper fruit cultivars at maturity stage (90 DAP)

3. Hormonal Treatments

Applying plant hormones or growth regulators, such as gibberellins can influence flowering, fruit set, and fruit quality by modulating plant physiological processes.

Singh and Singh (2019) studied the effect of gibberellic acid on growth, yield and quality parameters of chilli (*Capsicum annum* L.) and reportedvariations in capsaicinoid content (responsible for pungency) with respect to the concentration of GA_3 used. While moderate concentrations enhanced pungency and excessively high doses reduced the capsaicinoid level. Gibberellic acid application increased the ascorbic acid (vitamin C) content in the fruits, enhancing their nutritional value. The experiment consisted of different gibberellic acid treatments viz. 0 PPM- GA0, 50 PPM- GA1,100 PPM- GA2, and 150 PPM- GA3 along with control.It was observed that the application of GA3 @ 150 PPM was significantly superior over other treatments in terms of vitamin C content (98.49 mg/100 g).

Treatment	fresh weight of 10 fruits of chilli (g)	Number of fruits of chilli/plant	fruit yield/ plant of chilli (g)	fruit yield/ ha of chilli (t)	Vitamin-C content of chilli (mg/100g)
GA_0	69.88	78.12	447.47	18.62	96.66
GA_1	99.34	111.97	578.45	23.48	98.03
GA_2	105.65	118.64	610.83	24.57	98.46
GA_3	109.85	127.91	636.04	25.70	98.49
SEm±	0.50	0.55	1.17	0.08	0.10
CD(P=0.05)	1.65	1.85	3.91	0.25	0.30

Table 2. Effect gibberellic acid on yield and quality parameters of chilli

The influence of plant growth regulators on the growth, production, and quality of chilli (*Capsicum annuum*) was investigated by Chaudhary *et al*. (2006). Two well-known chilli cultivars, Jwala and Suryamukhi were used as the main plot treatments. Four growth regulators(2, 4-D at 2 ppm, NAA at 40 ppm, triacontanol at 5 ppm, and GA3 at 10 ppm) including water spray as the control were used as sub-plot treatments. The treatment GA3 at 10 ppm showed the highest ascorbic acid content. The ascorbic acid content of fruits increased dramatically (127.2 mg/100 g) when GA3 was used instead of conventional growth regulators. The increase in ascorbic acid by the application GA3 wasattributed eitherto increased ascorbic acid biosynthesis or for the protection of synthesized ascorbic acid from oxidation through the enzyme ascorbic acid oxidase. GA3 activates or upregulatesthe enzymes involved in the biosynthetic pathway of ascorbic acid, such as L-galactono-1,4-lactone dehydrogenase andthese enzymescatalyze the final step in the conversion of precursors into ascorbic acid. Gibberellic acid can also influence gene expression related to the biosynthesis of ascorbic acid, leading to increased enzyme production and activity.

Treatment	Number of fruits/plant	Fruit length (cm)	Number of seeds/fruit	Seed weight/ fruits (g)	Fruit yield (q/ha)	Ascorbic acid (mg/100g)
A. Varieties						
Jwala	100	6.5	51	0.19	171.5	116
Suryamukhi	109	5.6	60	0.2	191.2	101.6
S Em	1.1	0.1	0.6	0.01	4	1
LSD (P=0.05)	3.5	0.32	1.9	0.03	12.73	3.2
B. PGRs						
Control	83	5.9	54	0.16	152.9	98.1
2,4-D 2 ppm	124	6.3	61	0.24	214.6	111.6
NAA 40 ppm	107	6	54	0.17	177	89.5
Triacontanol 5 ppm	113	6.2	55	0.23	205.8	117.8
GA3 10 ppm	94	5.9	55	0.18	156.5	127.2
S Em	2.5	0.1	1.5	0.01	6.4	2.3
LSD (P=0.05)	7.35	0.25	4.24	0.03	18.77	6.63

Table 3. Effect of plant growth regulators(2, 4-D at 2 ppm, NAA at 40 ppm, triacontanol at 5 ppm and GA3 at 10 ppm)on the yield and yield attributes of chilli.

4. Nutrient Management: Inorganic and Organic Fertilizers

The type and amount of fertilizers used can impact the fruit quality. Nitrogen, phosphorus, and potassium are essential nutrients that influence plant growth, fruit size, and biochemical composition, including capsaicinoid levels. In addition to inorganic nutrients, organic fertilizers such as goat manure, can also improve soil health and provide slow release of nutrients, enhancing the fruit quality and potentially increasing capsaicinoid and carotenoid contents.

4.1 Inorganic Fertilizers

Altaf *et al.* (2019) studied the effect of NPK, organic manures and their combination on growth, yield and nutrient uptake of chilli (*Capsicum Annum* L.). NPK100%+FYM@8 ton per hectare resulted in increase in oleoresin yield and ascorbic acid content. The increase in ascorbic acid was attributed to improved nutrient status and enhanced photosynthetic activity, which support ascorbic acid biosynthesis. Oleoresin, a valuable extract containing capsaicinoids and carotenoids, is an important quality parameter for both flavor and nutritional value. Higher oleoresin content enhances the pungency and flavor profile of the chilies, making them more desirable for processing and consumption. Application of FYM in combination with NPK100% also resulted in increase in the uptake of nutrients (N, P, K, Ca, S, and Fe) when compared to the control and all other treatments. Increasing organic matter content could improve soil fertility, enhance nutrient cycling and facilitate sustained nutrient release.

a) b)

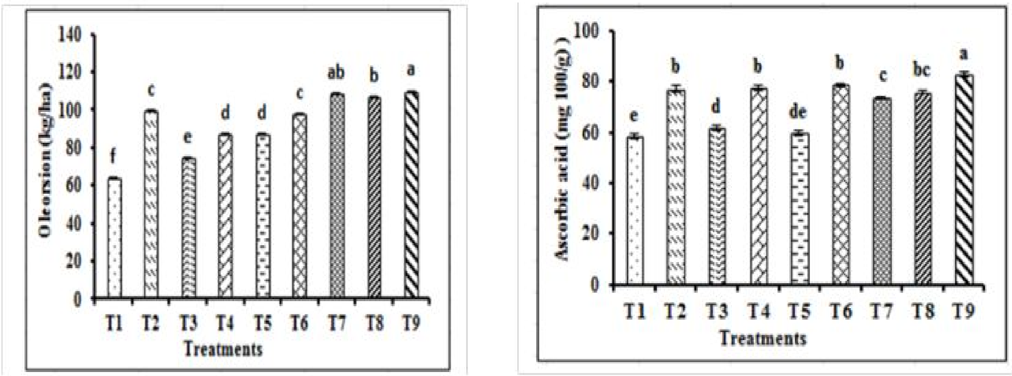


Figure 5. a)Oleoresin and b)Ascorbic acid yield of chili as affected by organic manures and NPK Fertilizer. Treatments=T, (T1; without NPK, Groundnut Cake (GC), Compost (C), Poultry Manure (PM), Vermicompost (VC) and FYM(Farm yard manure)- control, T2; N100%, T3; P100%, T4; K100%, T5; NPK100%+GC, T6; NPK100%+C, T7; NPK100%+PM, T8; NPK100%+VC, T9; NPK100%+FYM)

4.2 Organic Fertilizers

In 2017–2018, Gangadhar *et al*. (2020) carried out a field experiment at the Research and demonstration block of the Research Institute on Organic Farming (RIOF), UAS, GKVK, Bengaluru, on the effects of various organic manures and decomposers in combination on the growth, yield, and quality parameters of chillies (*Capsicum annuum* L.). Factor I comprised of organic materials such as sheep manure (SM), poultry manure (PM), vermicompost (VC), and farm yard manure (FYM). Factor II consisted of decomposers like Jeevamrutha (J), microbial consortia, and N: decomposer developed by NCOF(National Centre of Organic Farming).A Factorial Randomized Block Design experiment was set up with twelve treatments that were duplicated three times. FYM x Jeevamrutha (T1), FYM x Microbial consortia (T2), FYM x Decomposer (T3), Vermicompost x Jeevamrutha (T4), Vermicompost x Microbial consortia (T5), Vermicompost x Decomposer (T6), and Poultry (T7),Poultry manure x Jeevamrutha, T8: Poultry manure x Microbial consortia, T9: Poultry manure x Decomposer, T10: Sheep manure x Jeevamrutha, T11: Sheep manure x Microbial consortia and T12: Sheep manure x Decomposerwere the treatments. The application of vermicompost and Jeevamritha to various sources of organic manures, such as FYM, sheep manure, poultry manure, microbial consortia, and decomposer application, improved the quality parameters, such as ascorbic acid, TSS content, capsaicin (%), oleresin quantity, and shelf life of chilli. Chili had a much longer shelf life when combined application of vermicompost and jeevamrutha was used. Similarly Mbatha *et al*. (2021) reported that goat and poultry manure application increased Ca, Mg, K, P, and micro-nutrients content in sesamum (*S. alatum*) and the potential of goat manure to increase the nutrient contentwas more than that of the poultry manure.

Manures	Quality parameter during 2017							
	Ascorbic acid (mg 100 g^{-1})				Capsaicin (%)			
	Decomposers				Decomposers			
	J	C	N	Mean	J	C	N	Mean
FYM	121.64	116.95	113.21	117.27	10.84	10.42	10.09	10.45
VC	126.05	120.51	116.10	120.89	11.23	10.74	10.34	10.77
PM	125.20	119.23	115.20	119.87	11.15	10.62	10.26	10.68
SM	124.15	117.52	114.54	118.74	11.06	10.47	10.20	10.58
Mean	124.26	118.55	114.76		11.07	10.56	10.22	
	S. Em ±		CD (P = 0.05)		S. Em ±		CD (P = 0.05)	
M	3.176		12.256		0.283		NS	
D	2.750		8.254		0.245		NS	
M x D	5.501		NS		0.490		NS	
Manures	Quality parameter during 2017							
	Oleoresin quantity (%)				TSS (°Brix)			
	Decomposers				Decomposers			
	J	C	N	Mean	J	C	N	Mean
FYM	63.53	59.46	46.12	56.37	3.52	3.29	2.53	3.11
VC	68.45	64.30	57.91	63.56	3.80	3.51	3.15	3.49
PM	65.66	64.26	49.97	59.96	3.64	3.51	2.76	3.31
SM	64.20	62.04	47.43	57.89	3.56	3.44	2.62	3.21
Mean	65.46	62.52	50.36		3.63	3.44	2.77	
	S. Em ±		CD (P = 0.05)		S. Em ±		CD (P = 0.05)	
M	1.578		4.627		0.087		0.255	
D	1.366		4.007		0.075		0.221	
M x D	2.733		NS		0.151		NS	

CD at 5% NS - Non-Significant DAT- Days after Transplanting M - Manures
RDF: 125:75:63 kg N: P2O5:K2O ha^{-1} for N equivalent FYM application D - Decomposers
FYM - Farm yard manure VC - Vermicompost PM - Poultry manure SM - Sheep manure
J - Jeevamrutha C - Microbial Consortia N - Decomposer from NCOF

Table 4. Quality parameters of Chilli as influenced by application of organic manures, jeevamrutha and decomposers

Zhang *et al*. (2020) investigated the effect of several fertilizers based on biochar on the biological characteristics and financial advantages of pod pepper (*Capsicum annuum var. frutescens* L.). Biochar is a carbon-rich product obtained from the pyrolysis of organic material and is known for its potential to improve soil health and crop productivity. Biochar-based fertilizers have been frequently found as amendments to enhance crop yield and soil quality. Biochar improves soil physical properties such as porosity and water-holding capacity, which facilitate better nutrient uptake by plants.The addition of biochar stimulates soil microbial activity, enhance nutrient cycling and nutrient availability. In Guizhou Province, China, two field experiments were carried out in 2018 and 2019 to study the impacts of various biochar-based fertilizers on pod pepper yield, fruit quality, nutrient absorption and utilization, and economic advantages. The findings showed that, in comparison to traditional fertilization practices (TFP), the application of biochar-based fertilizers considerably raised the fruit quality and output. The experiment consisted of six treatments and three replications in a completely randomized block design . The six treatments were: No fertilizer (CK), traditional fertilization practice (TFP), biochar-based fertilizer (N-P2O5-K2O 12-6-10.8, carbon 10% (biochar-based fertilizer 1, B1), N-P2O5-K2O 12-6-10.8, carbon 20% (biochar-based fertilizer 2,B2), N-P2O5-K2O 12-6-10.8, carbon 30% (biochar-based fertilizer ,3B3), N-P2O5-K2O 12-6-10.8, carbon 40% (biochar-based fertilizer 4, B4)). biochar-based fertilizer 2 (N-P2O5- K2O 12-6-10.8, carbon 20%), biochar-based fertilizer 3 (N-P2O5-K2O 12-6-10.8, carbon 30%) and biochar-based fertilizer 4 (N-P2O5-K2O 12-6-10.8, carbon 40%). With the application of biochar-based fertilizer (B3), no significant effect was found on free amino acids and sugar content , while the content of vitamin C (Vc) in fresh pod pepper increased significantly. By the supply of biochar-based fertilizer, the N, P, and K accumulation increased by 19.0-34.8%, 9.4-28.3%, and 28.4-51.7% in 2018 and 6.7-38.9%, 15.0-55.0%, and 20.4-60.0% in 2019 as compared to the TFP treatment. The highest rates of N, P, and K accumulation were seen during both the years in the treatment of B3. The use of biochar-based fertilizers offers a sustainable alternative to conventional fertilizers by enhancing soil health and reducing environmental impacts. Biochar-based fertilizers enhance the efficiency of nutrient uptake, particularly for nitrogen (N), phosphorus (P), and potassium (K). This improved efficiency is due to the ability of biochar to retain nutrients and preventing leaching of nutrients mainly nitrogen.

Year	Treatments	Nitrate ($mg \cdot kg^{-1}$)	Vc ($mg \cdot 100\ g^{-1}$)	Reducing sugar ($g \cdot kg^{-1}$)	Free amino acid ($g \cdot kg^{-1}$)
2018	CK	90.4±1.4 bc	87.2±0.3 f	24.3±1.4 a	5.8±0.1 a
	TFP	92.9±0.4 a	90.2±0.3 e	23.4±0.2 a	5.8±0.1 a
	B1	92.8±1.2 a	92.0±0.3 d	23.7±0.5 a	5.7±0.2 a
	B2	90.7±0.7 b	93.7±0.2 c	23.5±0.9 a	5.6±0.1 a
	B3	89.0±0.2 c	103.3±0.4 a	22.8±1.8 a	5.8±0.2 a
	B4	91.2±1.3 ab	94.2±0.1 b	23.3±0.8 a	5.8±0.1 a
2019	CK	76.5±0.2 b	72.1±0.4 e	26.8±0.4 a	5.9±0.2 a
	TFP	86.9±0.8 a	85.3±0.4 d	26.5±0.4 a	5.9±0.1 a
	B1	76.9±0.9 b	85.9±0.3 d	27.0±0.7 a	5.9±0.2 a
	B2	75.0±0.4 c	93.0±0.7 c	28.1±0.6 a	5.8±0.1 a
	B3	72.0±0.4 d	98.4±0.4 a	27.6±0.5 a	5.9±0.1 a
	B4	66.8±0.5 e	94.8±0.6 b	27.1±0.6 a	5.9±0.1 a

Table 5. Effects of different biochar based treatments on quality of fresh pod pepper

5. Biotechnological approaches

5.10 Invitro culture

Many scientists have experimented with different methods or techniques to manipulate the tissues or cultures in vitro in order to increase the output of secondary metabolites. Between 1975 and 1985, distinct strategies to maximize growth and product production started to emerge. More recently, enhanced yield in plants or plant cell cultures became possible through genetic engineering and metabolic engineering (Verpoote*et al*. 1999). Various studies have been conducted over the past thirty years to improve the ability of in vitro cultured cells and tissues to produce capsaicinoids through manipulation of culture strategies. These strategies include immobilization of cells or tissue, feeding of precursors or biotransformation using intermediaries, treatments by elicitors, nutrient limitation, pH stress, osmotic stress, and light regime strategy.

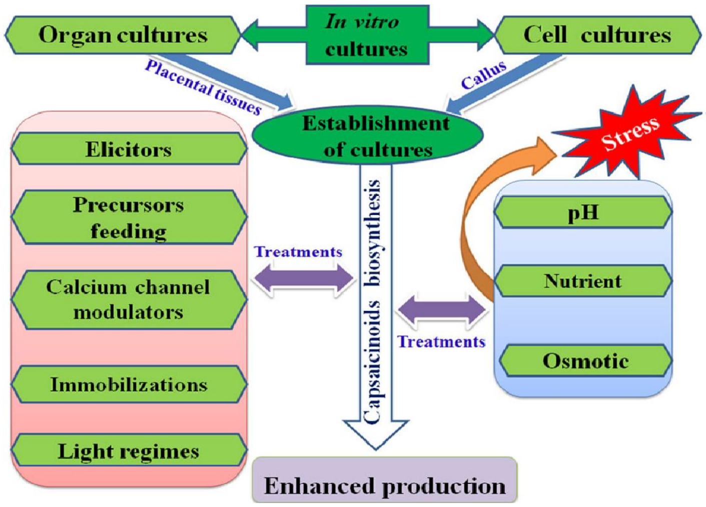


Figure 6. Invitro culture to enhance capsaicinKehie*et al*. (2015)

Very efficient substitute for producing capsaicin and its derivatives is in vitro synthesis by way of induction of callus and developing suspension cultures from chilli fruit.

The following steps are involved in in vitro production:- Callus induction from chilli fruits, the production of callus-derived cell suspension cultures, choosing cell lines with a high capsaicin yield, immobilization of plant cells to produce capsaicin, elicitation of culture system for augmenting the yield, capsaicinoid extraction from cell culture system, extraction of capsaicinoid from a system of cell culture,induction of callus from chilli fruit and creation of suspension cultures.The initial stage of producing capsaicin in vitro is called callus induction (Nisha *et al*., 2017). Callus development occurs when explants are implanted in plant tissue culture conditions supplemented with high auxin and low cytokinin concentrations (Umamaheswai and Lalitha, 2007).

Using cell-free extracts from the placentas of capsicum fruits, it was also shown that a-ketoisovalerate or a-ketoisocaproate is involved in the production of acyl moieties of capsaicinoid. Fujiwake*et al*. (1982) conducted more research on capsaicinoid production in a protoplast solution that was cultured at 38^{0} C for 120 minutes with either valine or phenylalanine. Protoplast suspension was evaluated by HPLC and scintillation spectrometry. The amino acid valine is the source of the branched fatty acid moiety of capsaicin, whereas phenylalanine serves as the precursor for the production of the vanillylamine moiety.

5.11 Application of Immobilized Cell System

Techniques for immobilization vary greatly, from membrane trapping to gel. For the trapping of plant cells, several gels are utilized, such as alginate and carrageen. Gels such as agarose, acrylamide, and agar are also utilized sometimes. Nonetheless, the gel-based approach is most effective for producing capsaicin. In order to produce secondary metabolites in tissue culture, most current method is Surface Immobilized Plant Cell (SPIC). In plant research and the development of plant cell culture, immobilized plant cells are extremely important. Some potential advantages are as follows: a) The immobilized cells are viable over a longer period both in the stationary and the reproducing stage, enabling to uphold cells for a long time period. b) Provides the prospective of easily attainable downstream processing (in case of secreted products). c) Dfferentiation gets promoted ,which is coupled with boosting secondary metabolism production. d) There is higher cell density allowing a smaller bioreactor, hence cutting down production costs and the risk of contamination e) Less shear sensitivity (especially with entrapped cells) f) Promotion of secondary metabolite accumulation, in some cases g) Flow-through reactors can be used enabling greater flow rates h) Decrease in fluid viscosity, which in cell suspension causes mixing and aeration problems (Brodelius*et al*.,1979).

Gel entrapment: The use of calcium alginate for plant cell immobilization was described by Brodelius*et al*. (1979). Since then, several gels have been employed for this purpose, either separately or in combination. The most popular gels are agarose, carrageenin, agar, and alginate.

5.12 **Entrapment in nets or foam:**

Polyurethane foam may entrap plant cells growing in liquid nutrient media in 1 cm^3 blocks. Gel blocks are continuously agitated while immersed in flasks containing cell suspension culture. The cells are first rinsed in and out of the blocks. But with time, the cells become imprisoned inside the internal matrix of blocks. Within the foam compartment, the cells merge to create bigger aggregates. The cubes are moved to a low-nutrient medium that promotes metabolic activity but inhibits cell proliferation after the blocks are fully filled. Immobilized cells stay contained in the foam and do not divide when put in the low growth media. Polyurethane matrices have no effect on the cell's capacity to survive. Any level of cell aggregation can be accommodated. They do not provide an external or internal barrier to the metabolites' diffusion. It was discovered that, under comparable circumstances, immobilized *Capsicum frutescens* cells produced more capsaicin than suspension-grown cells (Lindsey *et al.,* 1983).

Entrapment in hollow–fibre membranes: Moreover, hollow-fiber membranes are used to immobilize plant cells. Cell entrapment uses tubular fibers composed of silicone polycarbonate and cellulose acetate (Yeoman, 1987). The reaction vessel is filled to capacity with these fibers. Within the gaps between the fiber membranes, the cells become trapped. Different precursors and nutrients can travel through these gaps.

5.13 Influence of Elicitors on Capsaicinoids Biosynthesis

Elicitors can be classified as exogenous or endogenous based on where they came from, or they can be classified as biotic or abiotic based on their nature. Abiotic elicitors include substances produced from non-biological sources such as Cu2+ and Cd2+ions and physical conditions such as high pH. Materials with a biological origin are referred to as "biotic elicitors." These might be microorganism-generated chitin or glucans, or polysaccharides like pectin or cellulose obtained from plant cell walls. They also include glycoproteins or G-protein or intracellular proteins which are coupled to receptors and act by activating or inactivating the signal pathways (Tepic*et al*., 2008). Biologic in nature are both endogenous and exogenous elicitors. Exogenous elicitors are substances that are generated in the extracellular environment, such as polysaccharides, polyamines, and fatty acids, among others.
The impact of signaling chemicals, such as methyl jasmonate (MeJA) and salicylic acid (SA), on capsaicin induction in C. frutescens suspension cultures was documented by Sudha and Ravishankar (2003). It was discovered that while MeJA and SA alone increased capsaicin synthesis, their combination did not further increase capsaicin production. Overall capsaicin production was found to increase in the cultures of *Capsicum frutecense* when both methyl jasmonate (MeJA) and salicylic acid (SA) were supplied combined. However, the total buildup of capsaicin was reduced when the SA and MeJA were administered together (Sudha and Ravishankar, 2003). Ferulic acid doubled the amount of capsaicin present in immobilized cells (Ravishankar *et al*., 1999). Tyrosine and curdlan increased the output of capsaicin by 8.7 times (Ravishankar and Venkataraman, 1990). Following laminarin treatment, a 1.5–2 fold increase in capsaicin production was noted. According to Isleck et al. (2014), using cellulase for varying lengths of time increases capsaicin accumulation overall to varying degrees. But at greater concentrations and incubation time, capsaicin accumulation tends to decrease (Zenk, 1978).

Biotic elicitors	Abiotic elicitors
1. Synthesized by microbes and identified by plant cells (enzymes)	1. Physical or chemical in nature.
2. Synthesized by microbial activity on plant cell wall (fragments of pectin).	2. UV light.
3. Formed in the response to the activity of plant enzymes on microbial cell wall (chitosans, glucans).	3. Denatured proteins (RNase).
4. Compounds synthesized by plants in response to external agent.	4. Repetitive freezing–thawing sequence.
	5. Unwanted media constituents.
	6. High DNA affinity chemicals.
	7. Detergents
	8. Fungicides.
	9. Herbicides.

Table 6. Different biotic/abiotic elicitors Nisha *et al.* (2017)

In cell suspension cultures of *C. frutescens*, Prasad et *al*. (2006) examined the effects of biotic elicitors, such as aqueous mycelial extracts of **Rhizopus oligosporus** and *Aspergillus niger*, and abiotic elicitors, such as SA and MeJA, on the synthesis of capsaicinoids. When *R. oligosporus* mycelial extracts were added to cell-suspension cultures, there was a six-fold increase in capsaicinoids produced. Both elicited and nonelicited cell suspension cultures were used for the enzymatic tests of capsaicin synthase, keto acyl synthase, and caffeic acid O-methyl transferases. *R. oligosporus* mycelia extract treated suspension cultures overexpressed these enzymes, as reverse transcriptase polymerase chain reaction investigations revealed. Subsequent researcheshave demonstrated that the 8-methylnonenoic acid pool is essential in determining the efficacy of capsaicin levels (Prasad *et al*. 2006).

5.14. Strategies to increase yield of capsaicinoids through tissue culture

One important factor influencing the formation of secondary metabolites in plant tissue culture is the chemical composition of the culture medium (Misawa, 1985). One can raise the yield of the product by modifying specific elements of the culture environment. Plant cells use a heterotrophic form of nourishment when grown in cultures. They consist of additional inorganic nutrients for different physiological processes and simple sugars as a source of carbon. It has been demonstrated that in plant cell cultures, the amount of sucrose influences the build-up of secondary metabolites. The rise in secondary metabolites is most likely caused by the osmotic stress that sugar creates. In the measured concentration range of 4–12% (w/v), 8% (w/v) sucrose concentration was determined to be ideal for the accumulation of alkaloids (Knobloch and Berlin, 1980).
However, the type of plant and the class of alkaloids present also affect sucrose in the accumulation of secondary metabolites. Nitrogen sources in the form of ammonium and nitrate are present in all plant tissue culture medium. On the other hand, total nitrogen levels and the ratio of ammonium to nitrate also influence the quantity of secondary products that build. Research has shown that lower total nitrogen levels boost the output of anthraquinones in Morindacitrifolia, anthocyanins in Vitis species, and capsaicin in *Capsicum frutescens* (Yamakawa *et al*., 1983). Growth regulators are crucial for the build-up of secondary metabolites in suspension cultures of cells.
Secondary metabolite accumulation in cultivated plant cells is significantly influenced by the type, concentration, and ratio of auxin and cytokinin (Mantell and Smith, 1983). Auxin, or 2,4-D, can occasionally prevent the synthesis of secondary

metabolites. If this occurs, the output is increased by the medium that does not include 2,4-D or by substituting it with naphthalene acetic acid (NAA) or indole acetic acid (IAA). The way that cytokinins affect the formation of secondary metabolites varies depending on the species and kind of metabolite in question. Precursor feeding is a popular method to increase secondary metabolite production in cultured cells. Adding phenylalanine (precursor for vanillylamine) a constituent of capsaicin into the cell cultures of *Capsicum frutescens* enhanced the accumulation of capsaicin in the culture (Yeoman *et al.,* 1980)

6. CONCLUSION

Chilli is one of the important spice cum vegetable crop grown throughout the country. Quality attributes of chilli highly depends on various physiological parameters. The major quality parameters in chilli such as capsaicin, ascorbic acid, nutrient status, antioxidant content, sugar content etc. are highly influenced by physiological approaches such as stress induction (water and salt stress), hormonal treatment andnutrient management (organic and inorganic fertilizers).There exist a lot of scope to improve quality of chilliby genetic and biotechnological approaches also.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article.

REFERENCES

- Altaf, M. A., Shahid, R., Altaf, M. A., Ren, M. X., Tan, K., Xiang, W. Q., Qadir, A., Shakoor, A., and Altaf, M. M. 2019. Effect of NPK, organic manure and their combination on growth, yield and nutrient uptake of chilli (Capsicum annum L.). Hortic. Int. J. 3(1): 217-222.
- Brodelius, P., Deus, B., Mosbach, K., and Zenk, M. H. 1979. Immobilized plant cells for the production and transformation of natural products. Febs. Lett. 103(1): 93-97.
- Carrizo García, C., Barfuss, M. H., Sehr, E. M., Barboza, G. E., Samuel, R., Moscone, E. A., &Weigend, M. (2016). Phylogenetic relationships, diversification and expansion of chili peppers (Capsicum, Solanaceae). Annals of Botany, 118(1), 35-51
- Chaudhary, B. R., Sharma, M. D., Shakya, S. M., and Gautam, D. M. 2006. Effect of plant growth regulators on growth, yield and quality of chilli (Capsicum annuum L.) at Rampur, Chitwan. J. Inst. Agric. Animal Sci. 27(3): 65-68.
- Fujiwake, H., Suzuki, T., and Iwai, K. 1982. Capsaicinoid formation in the protoplast from the placenta of Capsicum fruits. Agric. Biol. Chem. 46(10): 2591-2592.
- Gangadhar, K., Devakumar, N., Vishwajith, and Lavanya, G. 2020. Growth, yield and quality parameters of chilli (*Capsicum annum* L.) as influenced by application of different organic manures and decomposers. *Intr. J. Chem. Studies* 8(1):473-482
- Hand, M. J., Nono, G. V., Tonfack, L. B., Youmbi, E., and Taffouo, V. D. 2021. Nutrient Composition, Antioxidant Components and Ascorbic Acid Content Response of Pepper Fruit (Capsicum annuum L.) Cultivars Grown under Salt Stress. Intr. J. Biol. 2(1): 43-70.
- Hill, T. A., Ashrafi, H., Reyes-Chin-Wo, S., Yao, J., Stoffel, K., & Van Deynze, A. (2013). Characterization of Capsicum annuum genetic diversity and population structure based on parallel polymorphism discovery with a 30K unigene pepper gene chip. *PLOS ONE, 8*(2), e56200.
- Jeeatid, N., Techawongstien, S., Suriharn, B., Chanthai, S. and Bosland, P.W., 2018. Influence of water stresses on capsaicinoid production in hot pepper (Capsicum chinense Jacq.) cultivars with different pungency levels. *Food Chemistry*, *245*, pp.792-797.
- Kehie, M., Kumaria, S., Tandon, P., and Ramchiary, N. 2015. Biotechnological advances on in vitro capsaicinoids biosynthesis in capsicum: A review. Phytochem. Rev. 14(2): 189-201.
- Knobloch, K. H. and Berlin, J. 1980. Influence of medium composition on the formation of secondary compounds in cell suspension cultures of Catharanthus roseus (L.) G. Don. Zeitschriftfuer. Natur. Forschung. 35(7-8): 551-556.
- Lindsey, K., Yeoman, M. M., Black, G. M., and Mavituna, F. 1983. A novel method for the immobilisation and culture of plant cells. Febs. Letters 155(1): 143-149.
- Mantell, S. H. and Smith, H. 1983. Cultural factors that influence secondary metabolite accumulations in plant cell and tissue cultures. In: Seminar series-society for experimental biology. Cambridge: Cambridge University, pp. 75-108.
- Misawa, M. 1985. Production of useful plant metabolites. Plant Cell Culture 6(2): 59-88.

- Narasimha, P.B.C., V. Kumar, H.B. Gururaj, R. Parimalan, P. Giridhar, and G.A. Ravishankar. 2006. Characterization of capsaicin synthase and identification of its gene (csy1) for pungency factor capsaicin in pepper (Capsicum sp.). Proc.Natl. Acad. Sci. USA 103:13315–13320.
- Naves, E. R., Valverde, M. E., Ramírez-Martínez, J. R., & Téllez, O. (2019). Capsaicinoids biosynthesis: A review of factors influencing the biosynthetic pathway and the use of biotechnology to enhance their production. *Biotechnology Advances, 37*(7), 107413.
- Nisha, S., Bora, A., Gogoi, H.K., Dwivedi, S.K. and Handique, P.J., 2021. A Study on the effect of different elicitors on capsaicin accumulation in cell suspension cultures of Capsicum assamicum (Bhut Jolokia).
- Prasad, N. B. C., Gururaj, H. B., Kumar, V., Giridhar, P., Parimalan, R., Sharma, A., and Ravishankar, G. A. 2006. Influence of 8-methyl-nonenoic acid on capsaicin biosynthesis in in-vivo and in-vitro cell cultures of Capsicum spp. J. Agric. Food Chem. 54(5): 1854-1859.
- Ravishankar, G. A. and Venkataraman, L. V. 1990. Food applications of plant cell cultures. Curr. Sci. 5(1): 914-920.
- Ravishankar, G. A., Bhyalakshmi, N., and Rao, R. S. 1999. Production of food additives. Biotechnol. Sec. Metabolites 5(1): 89-110.
- Ruiz-Lau, N., Medina-Lara, F., Minero-García, Y., Zamudio-Moreno, E., Guzmán-Antonio, A., Echevarría-Machado, I., and Martínez-Estévez, M. 2011. Water Deficit Affects the Accumulation of Capsaicinoids in Fruits of Capsicum chinense Jacq. Hort. Sci. 46(3): 487-492.
- Singh, S. and Singh, T. 2019. Effect of gibberellic acid on growth, yield and quality parameters of chilli (Capsicum annum L.). J. Pharmacognosy Phytochem. 8(2): 2021-2023.
- Stewart Jr, C., Kang, B. C., Liu, K., Mazourek, M., Moore, S. L., Yoo, E. Y., & Jahn, M. M. (2005). The Pun1 gene for pungency in pepper encodes a putative acyltransferase. *The Plant Journal, 42*(5), 675-688.
- Sudha, G. and Ravishankar, G. A. 2003. Influence of methyl jasmonate and salicylic acid in the enhancement of capsaicin production in cell suspension cultures of Capsicum frutescen Mill. Curr. Sci. 5(2): 1212-1217.
- Tepić, A. N., Dimić, G. R., Vujičić, B. L., Kevrešan, Ž. S., Varga, M., and Šumić, Z. M. 2008. Quality of commercial ground paprika and its oleoresins. Acta Periodica Technol. 3(9): 77-83.
- Umamaheswai, A. and Lalitha, V. 2007. In vitro effect of various growth hormones in Capsicum annum L. on the callus induction and production of Capsiacin. J. Plant Sci. 2(5): 545-551.
- Verpoorte, R., van der Heijden, R. T. J. M., Hoopen, T. H. J. G., and Memelink, J. 1999. Metabolic engineering of plant secondary metabolite pathways for the production of fine chemicals. Biotechnol. Letters 21(6): 467-479.
- Yamakawa, T., Kato, S., Ishida, K., Kodama, T., and Minoda, Y. 1983. Production of anthocyanins by Vitis cells in suspension culture. Agric. Biol. Chem. 47(10): 2185-2191.
- Yeoman, M. M. 1987. Techniques, characteristics, properties, and commercial potential of immobilized plant cells. Cell Cult. Somatic Cell Genetics Plants 3(1): 197-215.
- Yeoman, M. M., Meidzybrodzka, M. B., Lindsey, K., and McLauchlan, W. R.1980. The synthetic potential of cultured plant cells. Dev. Plant Biol. 2(1): 327–343.
- Zamljen, T., Medic, A., Hudina, M., Veberic, R. and Slatnar, A., 2022. Salt stress differentially affects the primary and secondary metabolism of peppers (Capsicum annuum L.) according to the genotype, fruit part, and salinity level. *Plants*, *11*(7), p.853.
- Zhang, Q., Zhang, F., Gong, C., Tan, X., Ren, Y., Yao, K., Zhang, Q., and Chi, Y. 2020. Physicochemical, microbial, and aroma characteristics of Chinese pickled red peppers (Capsicum annuum) with and without biofilm. RSC Adv. 10(11): 6609-6617.