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Studies on Seed Germination in Capsicum Seeds (Solan Bharpur) under Abiotic Stress

A Pathak[1], Arshia Kaur Virk[2], Shivani Sharma[3], Pancy Garg[4], Suman[5], Neetu Thakur[6]
[1, 2, 3, 4, 5, 6] *Department of Biotechnology, GGDSD College, Sector 32C, Chandigarh*

Abstract: ***Plants often experience abiotic stress like salinity, drought, high or low temperature, flooding, metal toxicity, ozone, UV-radiations, herbicides, etc., which pose serious threat to the crop production. Germination studies showed that heavy metal exposure caused a decrease in seed germination and at higher doses the seeds failed to germinate. This was confirmed by root length and shoot length. Ascorbic acid content was found to be decreased in all the treatment groups. However the phenol content was found to be decreased significantly with increasing concentrations of metals. There was considerable increase in phenolic content in water logging conditions. Metal treatment also led to an increase in catalase activity, thus showing that abiotic stress induced oxidative stress in the plants and antioxidants tend to neutralize this stress.***
Keywords: Copper; Cobalt; Zinc; Drought; Flood; Oxidative stress; Germination

I. INTRODUCTION

Plants are constantly confronted to both abiotic and biotic stresses that seriously reduce their productivity. Plant responses to these stresses are complex, induces a disruption in plant metabolism and involve numerous physiological, molecular, and cellular adaptations. Research on multiple stresses has been trying to simulate natural conditions, but in the field, conditions are not controlled, and one stress can strongly influence the primary stress defense response of the plants. Drought, high-salinity, heavy metal exposure, light, UV radiation, temperature extremes, and heat are major abiotic stresses that severely reduce the yield of food crops worldwide [1], [2], [3]. Thought to be integral to downstream defense/tolerance responses, the elevated levels of ROS are often associated with exposure to different biotic (e.g. pathogens or pests) and abiotic factors [4], [5], [6]. Overproduction of ROS leads to oxidative damage such as lipid peroxidation of cell membranes [5] or even cell death. In order to control ROS levels and protect cells from oxidative injury, plants possess both enzymes and non-enzymatic metabolites that may play a signifi cant role in ROS signalling in plants [7]. The harmful effects of ROS are prevented by the presence of antioxidants and antioxidative enzymes present in plant cells [8]. The ability to activate protective mechanisms, such as an increase in the activity of scavenging enzymes, is vital for oxidative stress tolerance The aim of the study was to determine the influence of heavy metals (Cu, Co and Zn), drought stress caused by treatment with mannitol and flood conditions on germination and seedling growth of Capsicum seeds (Solan Bharpur).

II. MATERIALS AND METHODS

A. Collection of Seeds

The seeds were collected from Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh

B. Sterilization of Seeds

The seeds were sterilized by first adding distilled water along with 2-3 drops of teepol. Then a piece of muslin cloth was cut to cover the beaker and the seeds were rinsed with distilled water 2-3 times. Then the seeds were soaked in bevistin solution (10%), for 5-10 minutes. The solution was drained and again the seeds were washed with distilled water 3-4 times.

C. Sowing of Seeds

All pots were labeled, and filled with soil collected from college garden. 15 seeds were sowed in each pot and were given different treatments as discussed below.

D. Chemical Treatments given to Seeds

The seeds were divided into 5 main groups and were given following treatment:

1) SET A (Control): Seeds in this group were treated with normal water.

2) *SET B ($CuSO_4$):* Seeds in this group were treated with different concentration of copper as CuSO4 mixed with distilled water: 1mM; 5mM; 10mM; 25mM; 50mM

3) *SET C ($CoCl_2$):* Seeds in this group were treated with different concentration of cobalt as $CoCl_2$ mixed with distilled water: 1mM; 5mM; 10mM; 25mM; 50mM

4) *SET D ($ZnSO_4$):* Seeds in this group were treated with different concentration of zinc as $ZnSO_4$ mixed with distilled water: 1mM; 5mM; 10mM; 25mM; 50mM

5) *SET E (Drought):* Seeds in this group were treated with different concentration of mannitol (mixed with distilled water): 50mM; 100mM; 200mM

6) *SET F (Flood):* Seeds in this group were treated with excess of water and water was allowed to stand as there was no outlet of water:

E. Observations under Consideration

The seeds started germinating after 11 days. The seeds in all pots were watered daily with specific solutions as prepared earlier. The number of seeds germinated the appearance of shoots and leaves were recorded daily for 21 days. Number of shoots, roots, leaves, root length and shoot length of all the above groups were measured at the end of 21 days.

F. Plants Extract Preparation

The extract of *in-vitro* raised control as well as treated plantlets was prepared in phosphate buffer (1M, pH-7). Before preparing the extracts, the plants were washed under running water and then washed 3-4 times with distilled water. 10% extract of plants belonging to different treated and control groups were prepared in phosphate buffer. The extracts were transferred to eppendorfs and then centrifuged at 2000rpm for 10 minutes, and the supernatant was used for various biochemical parameters.

G. Biochemical Parameters

Various biochemical tests were performed using different biochemical methods: Ascorbic acid; Phenol content; Reduced Glutathione levels; Catalase activity; Chlorophyll content

H. Statistical Analysis

All the experiments were done in triplicates on three different occasions. Values were expressed as mean ± SD. Statistical analysis was performed by students-t-test analysis. Comparisons were made with the control Vs treated groups.

III. RESULTS AND DISCUSSION

A. Germination Studies

Germination percentage clearly revealed that various treatments have their significant role on germination of seed. Increasing levels of copper, cobalt and zinc (Figure 1), decreased the germination of capsicum seeds.

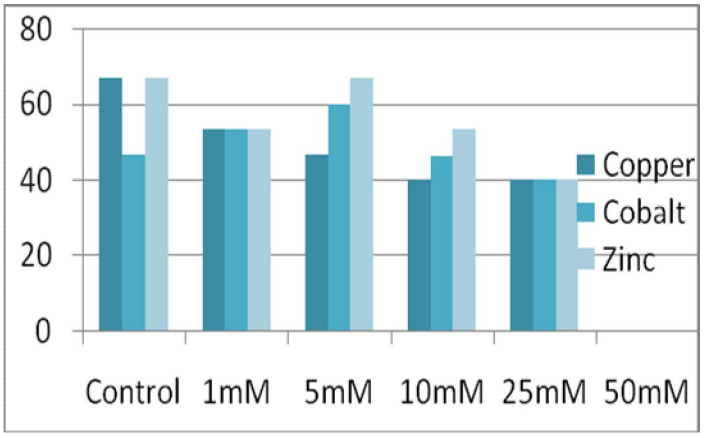


Fig 1.

Also flood (Figure 3) and drought (Figure 2) affected the rates of seed germination.

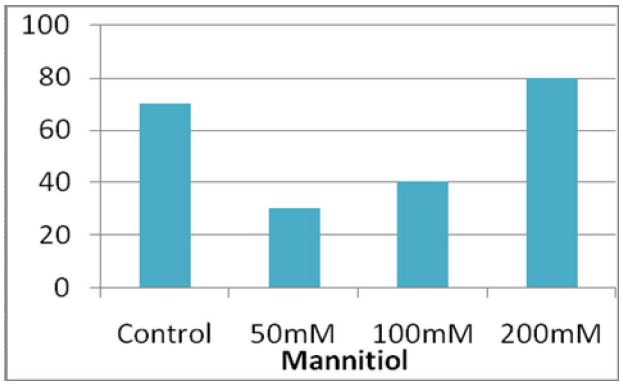


Fig 2.

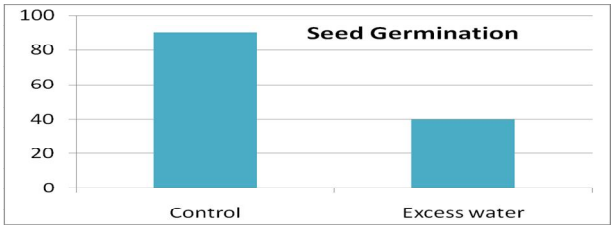


Fig 3.

The poor germination percentage and rate could result from the ionic toxicity which may be the cause of drastic effects of heavy metal salts on seed germination or it could be due to osmotic effect [9]. High level of copper concentrations has harmful effect on germination of Raphanus sativus L. var., Pusa chetki, Zea mays, Triticum aestivum [10], Vigna radiata [11], and Medicago sativa [12].The decrease in percentage of germination may also be due to loss of viability because of decreased energy generation by the embryo.

B. Root Length

When root length of control seeds was compared with $CuSO_4$ treated group, the root length of copper treated seeds was found to be more as compared to normal ones. As concentration of copper was increased, the root length was found to be increased heavily up to 5mM concentration and then it started decreasing (Figure 4). Similar trend was found in $CoCl_2$ treated seed. No roots emerged at 50mM concentration. This trend in root length suggests that at high level, this metal inhibit the roots growth, inhibiting cell division or cell elongation or combination of both leads to limited exploration of the soil volume for uptake and translocations of nutrients and water. Zinc treated groups also showed a significant decrease in root length (Figure 4).

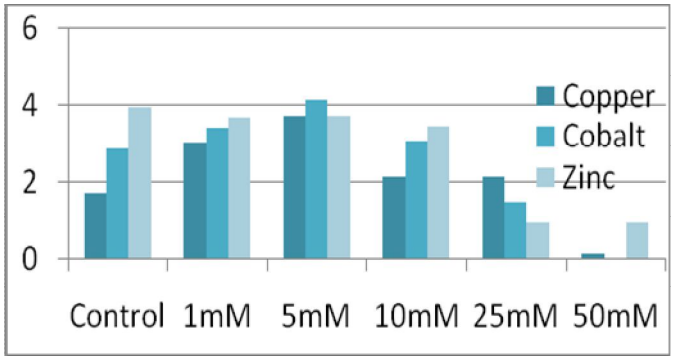


Fig 4

However, with increasing concentrations of mannitol, root length increases upto 200 mM concentrations (Figure 8). This shows that capsicum has some tendency to tolerate drought stress.

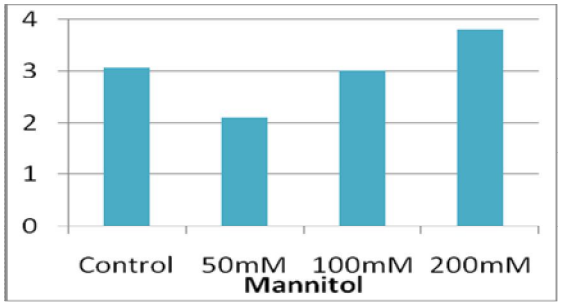


Fig 5

On the other hand, there was a decrease in root length in water logging conditions. This reduction in root growth in water logging conditions might be due to poor exchange of gases (Figure 6).

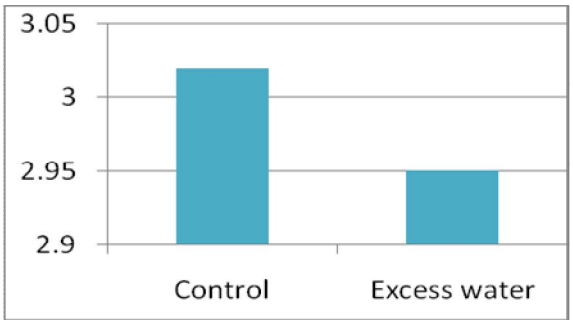


Fig 6

Heavy metals significantly affected root length. The inhibitory effect of metals on cell expansion and division might be due to inhibition of growth promoters primarily through blocking enzyme activation and lowering or direct blocking of cell division by intrusive with the cell membrane integrity [13]. These results of present study agreed with findings on the Cluster Bean [14] wheat [10] the Psyllium and on Mung bean .

C. Shoot Length

Shoot lengths of capsicum seeds were found to be decrease with the increasing concentration of copper at 5mM $CuSO_4$ and 25mM $CuSO_4$ treated group. And at very high concentration i.e. 50mM, there is no growth of shoots (Figure 7). Reduction in shoot and root lengths could be due to excess accumulation of salts in the cell wall, which modifies the metabolic activities negatively and limits the cell wall elasticity [15]. Shoot lengths of capsicum seeds were also found to be decreased with the increasing concentration of cobalt as well as zinc. Here also at very high concentration i.e. 50mM, there is no growth of shoots (Figure 7). The trend of decrease in shoot length occurs with increase in cobalt concentration suggested the toxicity of cobalt on capsicum seeds.

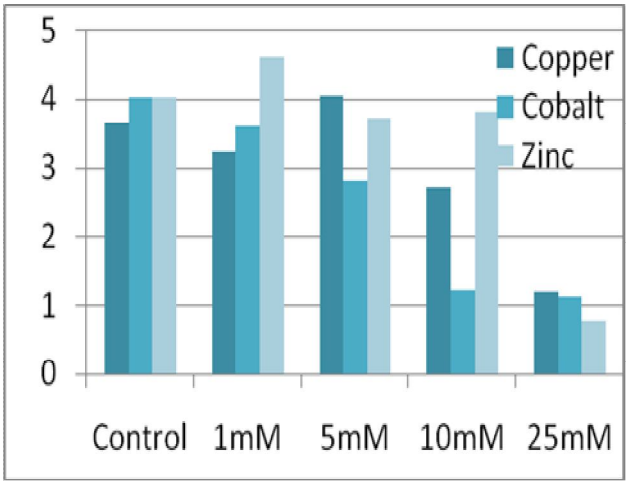


Fig 7

On the other hand, there was a significant increase of 72% with increasing concentrations of mannitol upto 200mM (Figure 8). But there was significant decrease in shoot length in water logging conditions. There was reduction in shoot length may be due to less uptake of nutrients in water logging conditions (Figure 9).

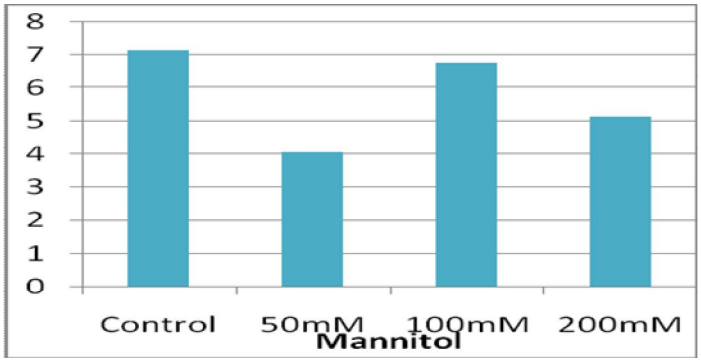


Fig 8

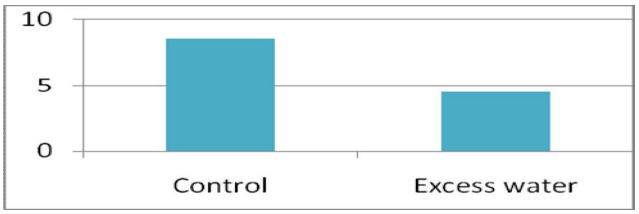


Fig 9

D. Ascorbic Acid Levels

Ascorbic acid concentration was found to be significantly decreased as concentration of copper, cobalt and zinc were increased (Table 1, 2, 3). Similar trend was noticed in drought conditions as well as water logging conditions as compared to the control group (Table 4, 5). However the phenol content was found to be decreased significantly with increasing concentrations of copper as well as cobalt (Table 1, 2). However, the phenol content was found to be increased at 1mM concentration of Zinc sulphate and decreased at 5mM concentration and again the content was found to be increased at the 25mM of zinc sulphate (Table 3). On the other hand, no significant change was observed in the phenol content at 50mM mannitol as compared to the control group. However at higher concentration, the phenol content was found to be increased (Table 4, 5). As compared with control, there was considerable increase in phenolic content in water logging conditions (Table 6).

E. Phenolic Content

High phenolic content may be due to their high tendency to chelate metal ions. Phenolics possess hydroxyl and carboxyl groups and are able to bind particularly iron and copper ions. The roots of many plants exposed to heavy metals exude high levels of phenolics. They may inactivate iron ions by chelating. This general chelating ability of phenolic compounds is probably related to the high nucleophilic character of the aromatic rings rather than specific chelating groups within the molecule. Phenols are synthesized in response to any stress to counteract the infection produced. Increase in the concentration of phenols in the present study suggests that these are synthesized to remove or neutralize the free radicals.

F. Catalase Activity

The catalase activity was found to be significantly increased at 1mM copper concentration and then decreased at higher copper concentration when compared to the control group (Table 1). In case of cobalt treatment, the catalase activity was found to be significantly increased as compared to the control group (Table 2). Zinc treatment also led to an increase in catalase activity when comparison was done with the control group (Table 3). However, mannitol treatment (Table 4) as well as flood condition caused a significant decrease in catalase activity (Table 5). Most authors reported increases in the catalase activity although in certain cases a decrease in the activity was also reported. Therefore, the exact mechanisms through which plants get rid of excess hydrogen peroxide is highly dependent not only of the species but on all the different exogenous factors affecting the experiment although at least one of these enzymes is usually found to increase its activity. In *B. juncea* under Cu stress an increase in APX and GPOD and a decrease in CAT, while in the same species as increase was also reported in SOD and APX and a decrease in CAT,

G. Chlorophyll Content

The chlorophyll content was found to be significantly increased at 1mM copper concentration and then it had to be decreased with increasing exposure of copper to the seeds (Table 1). Cobalt treatment, however, caused a significant decrease in total chlorophyll (Table 2). In case of zinc treatment, total chlorophyll content (Table 3) was found to be significantly increased at higher $ZnSO_4$ concentration as compared to the control group. Mannitol treatment, on the other hand , did not cause any change in the level of total chlorophyll(Table 4) when comparison was done with the control group . However ,water logging conditions caused a significant increase in the total chlorophyll (Table 5).The presence of heavy metals in toxic amounts is known to affect photosynthetic activity [16]. A reduction in chlorophyll synthesis (caused by the inhibition of the respective enzymes), increased chlorophyll destruction or affected element uptake (either by inhibition of the uptake or by competition with other heavy metals). Other causes for affected photosynthesis function include the inhibition of the Calvin cycle, reducing CO_2 fixation, the reduced aggregation of pigment protein complexes of the photosystems and general ROS induced damage to the chloroplasts [17], [18]. Most of the effects of heavy metals in chlorophyll are based on the measurement of the contents of this pigment and this leads to most authors justifying its decrease due to the inhibition of the enzymes responsible for its synthesis, although this conclusion is usually based on indirect evidence. [19] also reported reduced chlorophyll and carotenoid contents under Zn and Cd stress in *B. napus* plants, and justified the reduction due to the inhibition of enzymes leading to chlorophyll synthesis and the reduction in carotenoids due to the increased production of ROS. The chlorosis induced by Zn has often been attributed to an interference with Fe metabolism, although it can also be due to Mn deficiency. However Ebbs and Kochian reported decreased Fe and Mn content in shoots but little or no reduction in chlorophyll content.

H. Glutathione Levels

GSH levels were had to be significantly increased at 1mM copper concentration as compared to control ones. But at higher concentration GSH level were found to be significantly decreasing (Table 1). Cobalt treatment, however, caused a significant increase in GSH levels at all concentrations as compared to control group (Table 2). On the other hand increasing zinc concentration caused a decrease in GSH content as compared to the control group (Table 3). Mannitol (Table 4) and water logging conditions (Table 5) also caused a significant decrease in GSH content as compared with control.

Table I: Effect of Different Concentrations of Copper on Various Biochemical Parameters

Treatment	Ascorbic Acid (mg/ml)	Phenol (mg/ml)	Catalase (µmoles/min/mg of protein)	Total Chlorophyll	GSH (µmoles/mg of protein)
Control	0.60 ± 0.28	0.39 ± 0.001	13.64 ± 0.41	0.31±0.02	397.29 ± 016.99
1mM	0.26 ± 0.19	0.39 ± 0.005	28.21 ± 0.32	0.44±0.07	824.20 ± 120.63
5mM	0.15 ± 0.09	0.36 ± 0.02	15.50 ± 3.10	0.42±0.02	234.85 ± 150.02
10mM	0.20 ± 0.07	0.56 ± 0.009	03.54 ± 1.66	0.33±0.03	218.41±18.31
25mM	0.20 ± 0.07	0.48 ± 0.005	05.50 ± 1.11	0.23±0.02	225.73±19.12

Table II: Effect of Different Concentrations of Cobalt on Various Biochemical Parameters

Treatment	Ascorbic Acid (mg/ml)	Phenol (mg/ml)	Catalase (µmoles/min/mg of protein)	Total Chlorophyll	GSH (µmoles/mg of protein)
Control	2.00 ± 0.70	1.45 ± 0.08	3.10 ± 0.001	13.35 ± 8.45	223.9 ± 13.8
1mM	0.50 ± 0.04	0.50 ± 0.001	14.10 ± 1.68	0.96 ± 0.68	312.1 ± 13.0
5mM	0.60 ± 0.08	0.46 ± 0.008	12.54 ± 0.57	0.95 ± 0.56	297.3 ± 11.0
10mM	0.70 ± 0.08	0.54 ± 0.01	10.64 ± 2.56	0.89 ± 0.16	255.6 ± 18.6
25mM	0.50 ± 0.04	0.47 ± 0.05	8.24 ± 1.54	0.69 ± 0.24	207.5 ± 21.9

Table III: Effect of Different Concentrations of Zinc on Various Biochemical Parameters

Treatment	Ascorbic Acid (mg/ml)	Phenol (mg/ml)	Catalase (µmoles/min/mg of rotein)	Total Chlorophyll	GSH (µmoles/mg of protein)
Control	0.25 ± 0.01	25.40 ±1.92	0.03 ± 0.001	1.68 ± 0.12	335.97 ± 28.7
1mM	0.35 ± 0.05	36.60 ±4.05	0.12 ± 0.009	1.4 ± 0.61	290.4 ± 24.5
5mM	0.10 ± 0.001	57.57 ±0.17	0.16 ± 0.001	6.91 ± 0.01	140.4 ± 11.7
10mM	0.25 ± 0.07	48.92 ±0.65	0.16 ± 0.008	5.92 ± 0.02	156.88 ± 27.17
25mM	0.15 ± 0.007	42.43 ± 0.90	0.18 ± 0.003	1.14 ± 0.003	166.1 ± 20.24

Table IV: Effect of Different Concentrations of Mannitol on Various Biochemical Parameters

Treatment	Ascorbic Acid (mg/ml)	Phenol (mg/ml)	Catalase (µmoles/min/mg of protein)	Total Chlorophyll	GSH \(µmoles/mg of protein)
Control	0.26 ± 0.007	2.43 ± 0.41	2.18 ± 0.70	8.07±0.59	484.9 ± 21.6
50mM	0.26 ± 0.004	2.24 ± 0.20	2.02 ± 0.65	7.90±0.26	529.7 ± 24.6
100mM	0.26 ± 0.001	3.06 ± 0.65	1.15 ± 0.50	7.44±0.28	658.2 ± 16.6
200mM	0.19 ± 0.004	2.75 ± 0.34	0.14 ± 0.001	7.92±0.14	664.7 ± 10.3

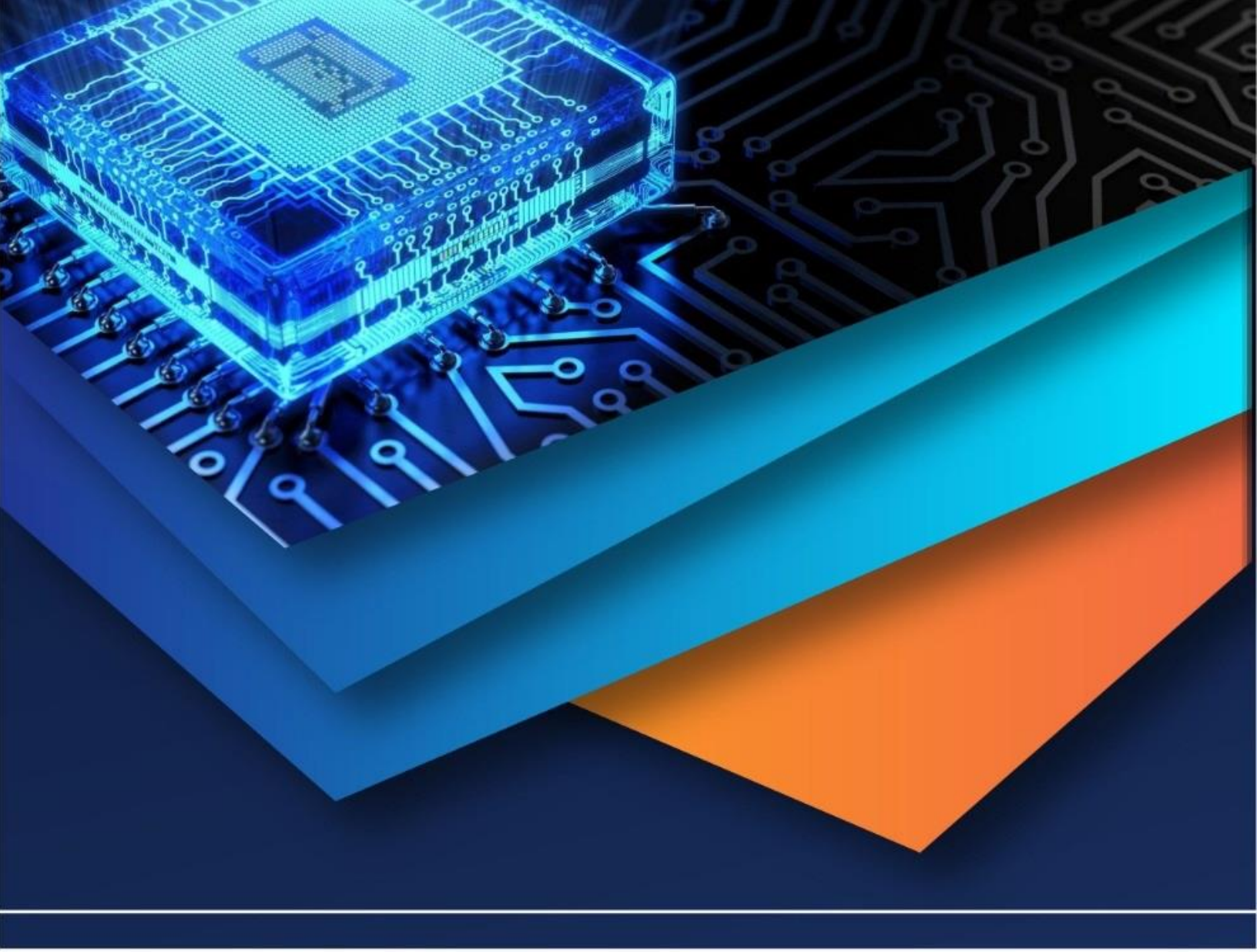
Table V: Effect of Excess of Water on Various Biochemical Parameters

Treatment	Ascorbic Acid(mg/ml)	Phenol (mg/ml)	Catalase (µmoles/min/mg of protein)	Total Chlorophyll	GSH (µmoles/mg of protein)
Control	0.14 ± 0.002	1.08±0.006	2.45 ± 0.001	7.10±0.41	380.8 ± 26.2
Test	0.17 ± 0.003	2.77±0.003	1.30 ± 0.006	5.04±0.64	251.1 ± 13.0

REFERENCES

[1] C. Aninbon, S. Jogloy, N. Vorasoot, A. Patanothai, S. Nuchadomrong, and T. Senawong. “Effect of end of season water deficit on phenolic compounds in peanut genotypes with different levels of resistance to drought,” Food Chem, vol 196., pp 123-9, Apr. 2016
[2] G. Kaur, S. Kumar, H. Nayyar, and H.D. Upadhyaya. “Cold stress injury during the pod- fi lling phase in chickpea (Cicer arietinum L.): effects on quantitative and qualitative components of seeds,” J Agron Crop Sci, vol. 194, pp. 457–464, 20
[3] P. Thakur, S. Kumar, J.A. Malik, J.D. Berger, and H. Nayyar . “Cold stress effects on reproductive development in grain crops: an overview,” Environ Exp Bot vol. 67, pp. 429–443, 20
[4] S. Neill, R. Barros, J. Bright, R. Desikan, J. Hancock, J. Harrison, P. Morris, D. Ribeiro, and I. Wilson. “Nitric oxide, stomatal closure, and abiotic stress,”*Journal of Experimental Botany*, vol. 59(2), pp. 165-176, Feb 20
[5] J. A. Imlay. “Pathways of oxidative damage,” Annual Review of Microbiology, vol. 57(1), pp. 395-418, 2003
[6] J.Einset, and E. Nielsen. “Membrane-trafficking RabA4c involved in the effect of glycine betaine on recovery from chilling stress in Arabidopsis,” , vol. 130(4), pp. 511-518, 2007.
[7] E. Vranová, D. Inzé, and F. V. Breusegem. “Signal transduction during oxidative stress,” J Exp Bot, vol. 53, pp. 1227–1236, 2002.
[8] R. Desikan, M-K. Cheung, J. Bright, D. Henson, J.T. Hancock, and S. J. Neill. “ABA, hydrogen peroxide and nitric oxide signaling in stomatal guard cells’” J Exp Bot, vol. 55, pp. 205–212, 2004
[9] S. S. Shaukat, M. Mohammad, and Z. S. Siddiqui. “Effect of cadmium, chromium and lead on seed germination, early seedling growth and phenolic contents of Parkinsonia aculeata L. and Pennisetum americanum (L.) Schumann,” Pakistan Journal of Biological Sciences vol. 2(4), pp. 1307.1313, 1999.
[10] Singh, S. N.and R. D. Tripahti (Eds), Biotechnological approaches to improve phytoremediation efficiency for environment contaminants. In: Environmental bioremediation technologies: Springer, 2007, pp. 223-
[11] C. Hema, and A. Subramani. “Phyto toxic effect of copper and chromium on seed germination percentage of Vigna radiata.L,” Int J Cur Res Rev, vol. 5(5), pp. 95-100, 201
[12] Jadia, and Fulekar.”Phytoremediation of heavy metals: recent techniques,” African Journal of Biotechnology, vol. 8(6), pp. 921–928, 2009.

[13] O. Munzuroglu, N. Gur." The effects of metals on the pollen germination and pollen tube growth of apples (Malus sylvestris miller cv. Golden)," Tr J Biol, vol. 24, pp. 677–684, 2000.
[14] R. Manivasagaperumal., et al. "Effect of zinc on germination, seedling growth and biochemical content of cluster bean (Cyamopsis tetragonoloba (L.) Taub)," Current Botany, vol. 2(5), pp. 11-15, 2001
[15] Naseer, Shazia, Nisar, and Ashraf. "Effect of salt stress on germination and seedling growth of barley (Hordeum vulgare L.).," Pak. J. Biol. Sci, vol. 4(3), pp. 359-360.2001.
[16] B. Mysliwa-Kurdziel, M. N. V. Prasad, and K. Strzalka. "Photosynthesis in heavy metal stressed plants," Springer, pp. 146–181, 2004
[17] M. Mobin.,N. A. Khan N.A. "Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress," J. Plant Physiol, vol. 164, pp. 601–610, 2007
[18] A. B. Ghnaya, G. Charles, a. Hourmant, J. Ben Hamida, and M. Branchard . " Physiological behaviour of four rapeseed cultivar (*Brassicanapus* L.)," C. R. Biol., vol. 332, pp. 363–370, 2009.
[19] S. D. Ebbs, and L. V. Kochian., "Toxicity of zinc and copper to brassica species: Implications for phytoremediation," J. Environ. Qual,



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