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MITIGATION OF ABIOTIC STRESS IN WATER DEVOURABLE PLANTS USING *ARBUSCULAR MYCORRHIZAL* FUNGI AND PLANT GROWTH PROMOTING BACTERIA

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ABSTRACT

The Amalgamation of *Arbuscular mycorrhizal* fungi (AMF) and plant growth-promoting bacteria (PGPB) provides so many benefits to plants under stress conditions (salinity and heavy metals). This combination leads to an increase in the soil structure, aggregation, and yield productivity. The effect of *Aspergillus flavus* (AMF) and *Pseudomonas fluorescens* (PGPB) in Basil plant is to ameliorate their growth, phosphorous content and stress tolerance. As an added value, the presence of *mycorrhizal* fungus stimulated in all growth parameters and also comparing with both treated and non-mycorrhizal treated plants. In addition, the abiotic stress also declines the relative water content (RWC) so when treated with *A. flavus* mitigates the deleterious effects on plants under stress conditions. A combination of AMF and PGPB enhanced morphological parameters like plant biomass, photosynthetic pigment, relative water content (RWC) and levels of hydrogen peroxide. Finally, these results suggested that amalgamation of AMF and PGPB increased the photosynthetic rate under stress conditions.

KEYWORDS: Basil, *Aspergillus flavus*, *Pseudomonas fluorescens*, Plant parameters, Mitigation of stress.

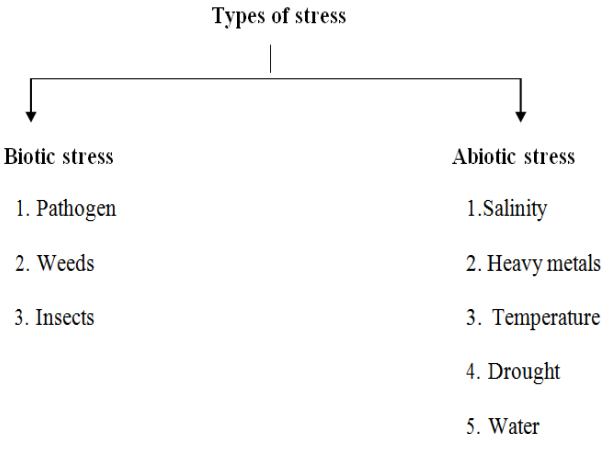
1. INTRODUCTION

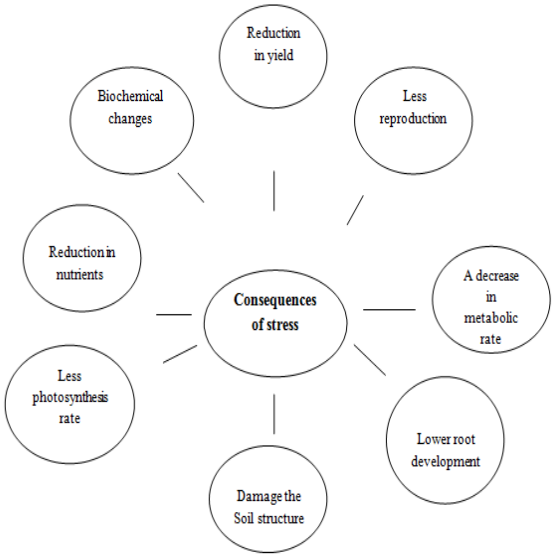
Plants are mainly multicellular, photosynthetic eukaryotes of the kingdom Plantae. They are treated as one of two kingdoms including all living things that were not animals, and all algae and fungi were treated as plants. Green plants uptake most of their energy from sunlight through photosynthesis by primary chloroplasts. Their chloroplasts contain chlorophylls a and b, which gives them their green color (Cavalier-Smith T and Chao EE, 2020).

Basil (*Ocimum basilicum*) is a herb that belongs to the Lamiaceae family and also known as great basil. Another name of Basil is Saint Joseph's Wort and also belongs to the mint family. It is native to tropical regions from central Africa to Southeast Asia and there are various types of basil based on their taste and smell (Balakrishnan Purushothaman *et al*., 2018).

Plant stress refers to non-ideal growth conditions that affect growth, development or productivity of plants. It leads to insufficient metabolism, reproduction, root development or growth of the plant, gene expression, and crop yield. Plant stresses are caused by a natural disaster such as drought and wind or by anthropogenic (Verma and Nizam, 2013).

There are two types of stress which affect plant growth such as biotic and abiotic stresses. Both are made in plants by stress-causing factors.





The mitigation of stress is an important research area with the increasingly ubiquitous demands of modern lifestyles. With the advent of physiological measures for the identification of stress, recent research into applications of these measures has opened new horizons for the objective treatment of these stress. (Ahmad Rauf Subhani *et al*., 2018). Here we are using the source of microorganisms such as fungi and bacteria to mitigate the stress.

Arbuscular mycorrhizal are soil-borne fungi living microorganisms that can significantly improve the plant nutrients and also uptake the resistance to several abiotic stress factors. It maintains a symbiotic relationship with the plants. It also helps to increase plant growth. (Sun *et al*., 2018). Most of the plants have the potential to develop a symbiotic relationship with *Arbuscular mycorrhizal* fungi (AMF). The effect of symbiotic relationship with plants is that the transfer of nutrients from the soil to plants.

By symbiotic relationship of AMF with the root system of plant results in high yield production even under salinity and contaminated soil by heavy metal. The promotion of plant growth is enhanced by the release of secondary metabolites (gibberellins, auxins, and cytokinin) that are stimulated by the AMF. Plant-microbes symbiosis also influences the stomatal regulation, enhances the water uptake at low soil moisture level, increases the photosynthetic activity, proline and carbohydrate accumulation and increases the nutritional level (Lamia Zarik *et al*.,2016).

Plant growth-promoting bacteria (PGPB) are bacteria that can enhance plant growth and protect plants from disease and abiotic stresses through a wide variety of mechanisms; those that establish close associations with plants, such as the endophytes, could be more successful in plant growth promotion. In addition to PGPB, some fungi have also been demonstrated to promote plant growth. Apart from improving crop yields, some biofertilizers also control various plant pathogens (Olanrewaju *et al*., 2017). It plays a major role in the induced systemic resistance (ISR), and it can promote plant growth without any harmful effects.

A bacterium which increases the plant growth, nutrient availability and metabolic rate are called the plant growth-promoting bacteria (PGPB). There are two types of plant growth promoters-direct and indirect. Direct plant growth promoters contribute the plant growth directly without external sources. It is classified as biofertilizers, rhizoremediators, phytostimulators or stress controllers. Biofertilizer increases the plant growth via nitrogen fixation, phosphorous solubilization, potassium solubilization, sequestering iron and sulfur oxidation (Siddiqui and Singh, 2005). Rhizo remediation helps the plant to grow even in the polluted soil. Phyto stimulators trigger the production of phytohormones such as auxin, cytokinins, gibberellins (GAs), abscisic acid (ABA), ethylene (ET), brassinosteroids (BRs), jasmonic acid (JA), salicylic acid (SA) and strigolactones (SLs). Indirect plant growth promoters do not contribute the plant growth directly.

It improves the growth through Antibiosis, lytic enzyme production, competition for nutrients, induced systemic resistance (ISR) and volatile organic compounds (VOCs) (Baliah and David *et al*., 2018).

2. MATERIALS AND METHODS

2.1 Collection of soil

A soil sample was collected from the nearby area and it was spread on a sheet to dry for a few days. Then the soil was separated into four quarters and we eliminated the two quarters. The rest two quarters were sent for analysis such as micronutrients, primary nutrients, secondary nutrients, pH, electrical conductivity and organic content.

2.2 Estimation of nutrients, pH and electrical conductance of the soil

About 100 g of soil was sent for analysis to A.M.M. Murugappa Chettiar Research Center, Taramani, Chennai. Then we got an estimation result that how much amount of nutrients are present in our soil and also pH and electrical conductance of soil.

2.3 Sowing of seeds and plant cultivation

We have chosen water devourable plant Basil. The soil pH was analyzed before sowing the seeds. Then we collected the seeds of the plants and it was sown in our desired soil. We waited patiently for the plants to sprout out.

2.4 Stress creation

After the plant has attained its exponential stage, we created the stress such as salinity (NaCl) and heavy metals (Cd and Cr) on the plants (Abeer Hashem *et al*., 2018; Mayada Sabra *et al*., 2018).

2.4.1 Salinity stress

Table 1 Stress creation 1.

S. NO	Days	Salt stress created by us (pH)	The quantity we sprayed (ml)	Maintained Ph for Basil
1	Day 1	12.0	10	8.40
2	Day 2	12.0	10	8.54
3	Day 3	12.0	10	8.58
4	Day 4	12.0	10	8.71

Table 2: Stress creation 2.

S. NO	Days	Salt stress created by us (pH)	The quantity we sprayed (ml)	Maintained pH for Basil
1	Day 1	13.0	10	9.60
2	Day 2	13.0	10	9.44
3	Day 3	13.0	10	9.57
4	Day 4	13.0	10	9.20

2.4.2 Heavy metal stress

Table 3: Stress creation 3.

S.NO	Days	Cd we sprayed (ml)	Cr we sprayed (ml)	Both Cd and Cr (ml)
1	Day 1	25	25	25
2	Day 2	25	25	25
3	Day 3	25	25	25
4	Day 4	25	25	25

2.5 Collection of fungi and bacteria

Aspergillus flavus fungi were collected from the National Culture Collection of Pathogenic Fungi (NCCPF), Chandigarh. It was stored at 4 ^{o}C and subcultured on the plate containing Potato dextrose agar that is the major source for the fungi growth. It took 5 days for the complete growth of the fungus. Bacteria (P.*fluorescens*) were subcultured in nutrient broth and kept it in a shaker for overnight to get the better of its growth. Later, it was stored at 4 ^{o}C until further uses.

Table 4: Composition of nutrient broth.

S.NO	Component	Composition(g/l)
1	Peptone	10
2	Yeast extract	10
3	NaCl	5

2.6 Inoculation of fungi and bacteria in plants

After stress creation on the plant, the growth of the plant started to shrink which shows there were no nutrients and minerals to survive. To overcome this problem, we inoculated the mixed culture of fungi and bacteria to regain its growth.

2.7 Harvesting

We gave a particular time to plants for microbes (AMF and PGPB) adaptation on the root of the plant after the microbes inoculation. When the plants attained their complete growth, they were moved for harvesting.

2.8 Preparation of plant extract

After the completion of the growth period, the different parts of the plants (root, stem, and leaves) were separated. They were washed with fresh water to remove the soil particles and were air-dried using hot air oven. The plant parts were turned into powdered form. The powder was mixed with an ethanolic solvent in the proportion (1:10) for the extraction of plant components. It was filtered to remove the impurities in the plant extract. Then it was centrifuged at 3,000 rpm for 5 minutes and later it was dried by evaporation. The dried extract was collected and suspended in Dimethyl sulfoxide (DMSO) in the proportion (10 mg/ml) and stored at -20 ^{o}C (Arti Goggi *et al*., 2017).

(a) (b) (c) (d) (e) (f)

Fig. 1: Treated and non-treated plants (a) pH 8 maintained (b) pH 9 maintained (c) Heavy metal (cd) consumed plant (d) Heavy metal (cr) consumed plant (e) Heavy metal (cd-cr) consumed plant (f) Control.

2.9 Identification of morphological parameters

There is some reduction in the root, shoot length and leaf area in *Ocimum basilicum* plant under salinity and heavy metal stress. To overcome these problems, we step forward to give a combined application of *Arbuscular mycorrhizal fungi* and plant growth-promoting bacteria to the plant (S. El Kinany *et al*., 2019).

2.10 Estimation of physical parameters

2.10.1 Relative water content

Expanded leaves were collected from the plants and they were punched into discs. Fresh weight of these leaf discs was measured and the same leaf discs were floated on water for 4 hours, and turgid weight was dogged. Then it was kept in the oven at 85 °C to record the dry weight. Calculation of the leaf water content was done using the following formula: (Aliabadi Farahani *et al*., 2008).

RWC = Fresh Weight-Dry weight × 100 Turgid Weight-Dry weight

2.11 Biochemical test

2.11.1 Plant pigment analysis

The content of photosynthetic pigment (chlorophyll, xanthophylls, and carotenoids) was extracted from the plant sample in 80% ethanol. The sample was centrifuged at 3000 rpm and the optical densities of supernatant were recorded at 480,640,663 nm with a spectrophotometer. The chlorophyll, xanthophylls, and carotenoids contents were calculated according to the following equations: (Delia-Gabriela Dumbrava *et al*., 2012).

Chl a = 12.21. (A_{663}) – 2.81. (A_{646})
Chl b = 20.13. (A_{646}) – 5.03. (A_{663}) Chl total = 17.32. (A_{646}) + 7.18. (A_{663})
Xanthophyll and Carotenoids = [(1000. A_{470}) - (3,27 Chl a) - (1,04 Chl b)] /229

Where:
Chl a – chlorophyll a, in mg/l, Chl b – chlorophyll b, in mg/l,
Chl total – total chlorophylls content, in mg/l, A663 – sample absorbance at 663 nm,
A646 – sample absorbance at 646 nm, A470 – sample absorbance at 470 nm.

2.12 Determination of hydrogen peroxide

Fresh tissue was homogenized and it was mixed with Trichloroacetic acid (TCA). Then it was centrifuged at 12,000 rpm for 15 minutes. The pellet was removed and the supernatant was collected in a microfuge tube. An equal volume of potassium phosphate buffer and potassium iodide was added to the supernatant containing a microfuge tube. Vortex the tube for proper mixing and the optical density was recorded at 390 nm (Abeer Hashem *et al*., 2019).

2.13 Statistical analysis

Statistical significance between the control and treatments were calculated by one-way ANOVA to determine differences in plant characteristics (Lamia Zarik *et al*., 2016).

3. RESULTS

3.1 Culturing of fungi

Desired microorganisms were grown under the nutrient media containing agar as a solidifying agent. Mainly Potato dextrose agar (PDA) present in the culture media that are enriched with nutrients and minimal agars require the addition of relevant nutrients such as a carbon and nitrogen source. In addition to nutrients essential for growth, various other components can be introduced into the agar media. Then, we inoculated the fungi culture that we need on the nutrient media. We observed the formation of fungi on the plate after the incubation.

Fig. 2: Cultivation of A.flavus.

3.2 Soil tests

Soil tests are used to measure the nutrients present in the soil that are necessary for plant growth. There are a lot of nutrients present in our soil.

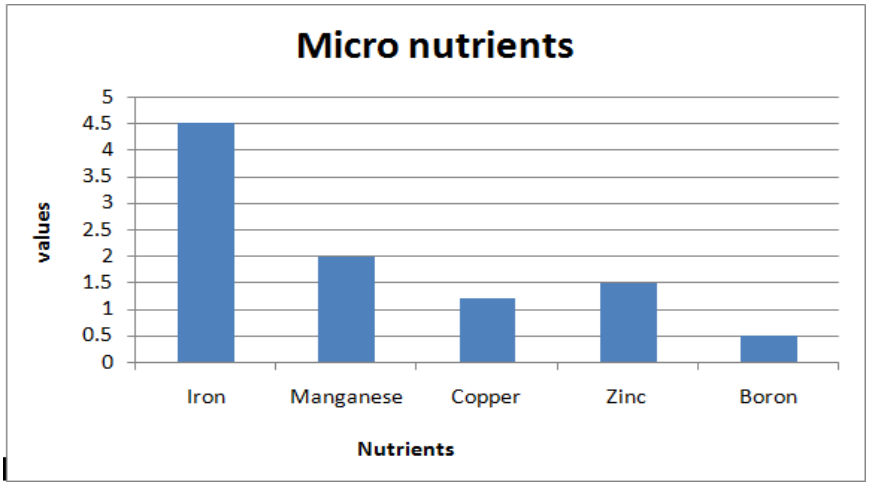


Fig. 3: Representation of micronutrients.

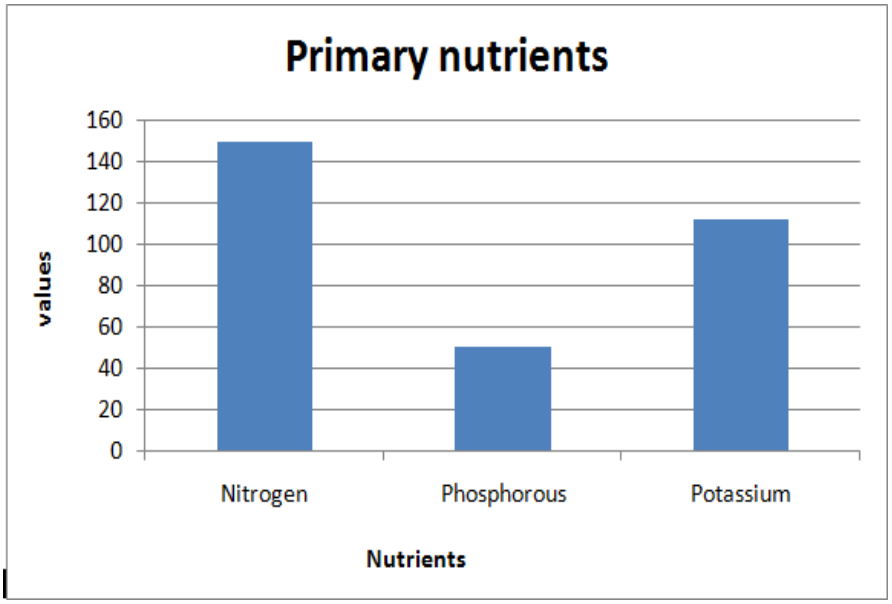


Fig. 4: Representation of primary nutrients.

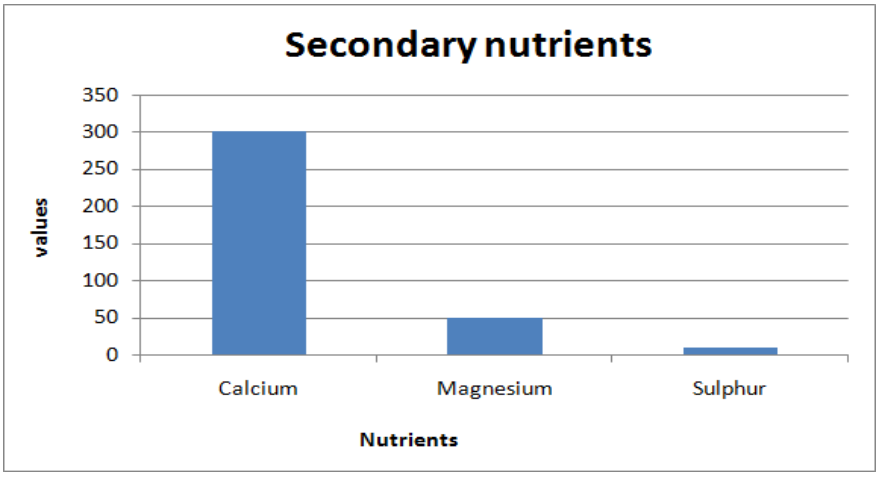


Fig. 5: Representation of secondary nutrients.

3.3 Effect of inoculation and abiotic stress on morphological parameter

There is some reduction in the root, shoot length and leaf area in *Ocimum basilicum* plant under salinity and heavy metal stress. To overcome these problems we step forward to give a combined application of *Arbuscular mycorrhizal* fungi and plant growth-promoting bacteria to the plant. After which we moved for morphological

parameter measurement. We observed that the application of AMF and PGPB stimulated plant growth independently under different stress conditions. Mycorrhized plants endorsed the effective growth than non inoculated plant. However, the application of AMF and PGPB mitigated abiotic stress. A combination of AMF and PGPB inoculation improved the morphological traits like root, shoot length and leaf area under different stress conditions.

There is a higher length of root and shoot in mycorrhized plants than in control plants. The significant reduction happened in morphological traits in the non inoculated plants. Overall, abiotic stress (salinity and heavy metal stress) declined shoot, root, leaves diameter. However, mycorrhized plants were not as much affected by abiotic stress compared to non-mycorrhized plants. The result in table 7 shown the morphological parameters of plants.

Table 5: Effect of inoculation and abiotic stress on morphological parameter.

S.NO	Morphological Parameters	Untreated plant (cm)	Treated plants (cm)				
		Control	Cd	Cr	Cd-Cr	pH-9	pH-10
1	Stem length	8.1	**10.5**	**10.2**	**10.8**	**10.45**	**10.1**
2	Leaf length	6.2	**2.7**	**6.8**	**7.2**	**7.25**	**7.1**
3	Leaf width	2.4	**2.9**	**2.9**	**3.1**	**2.8**	**2.6**
4	Root length	6.1	**6.8**	**7.2**	**6.92**	**6.85**	**6.7**

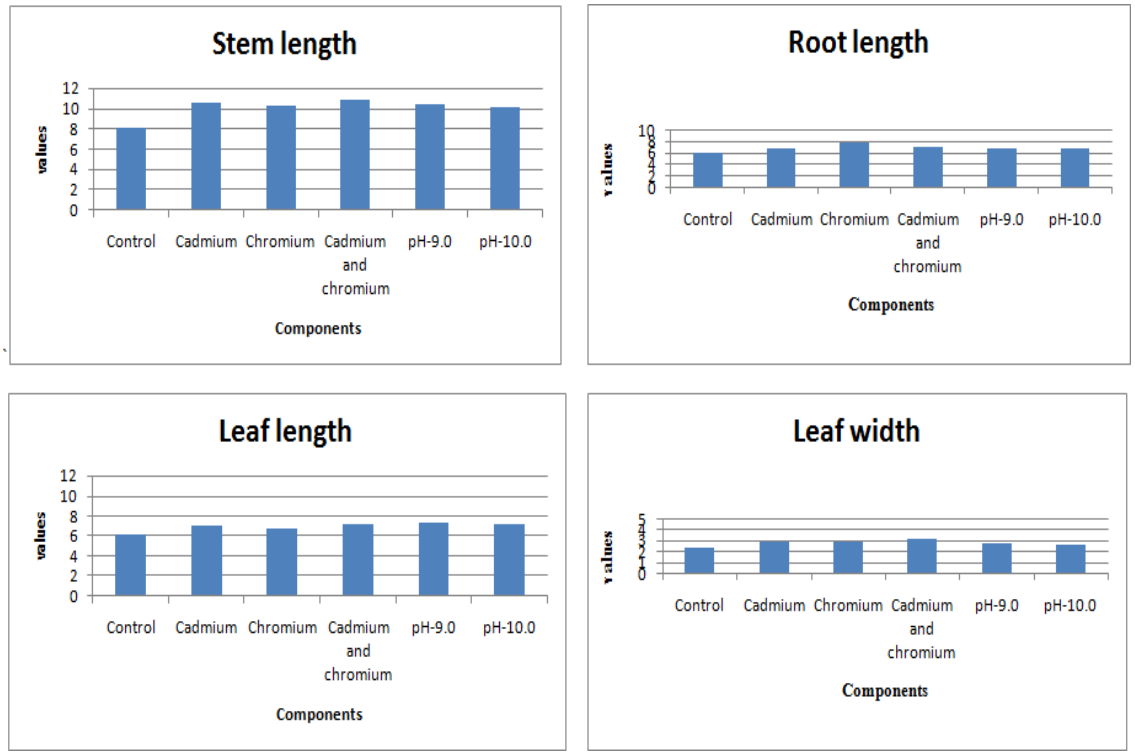


Fig. 6: Representation of morphological parameters.

Table 6: The result of one way ANOVA on the effect of inoculation and abiotic stress on morphological parameter.

ANOVA: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
8.1	7	122.3333	17.47619	354.9904		
6.2	7	83.825	11.975	170.101		
2.4	7	34	4.857143	27.76202		
6.1	7	81.80167	11.68595	162.2489		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	560.7182	3	186.9061	1.045479	0.39047	3.008787
Within Groups	4290.614	24	178.7756			

3.4 Effect of inoculation and abiotic stress on relative leaf water content

Owing to abiotic stress creation on the plants, relative water content (RWC) of that plant moved to diminish. By the combined application of microbes (AMF and PGPB), RWC of plants got increased slowly but surely. High significant RWC of leaves was attained compared to control treatment due to the inoculation of microbes on the plants. RWC of leaves was drastically affected without mycorrhizal inoculation. At first, we look frontward to determine the fresh weight of the leaves. Afterward, the same leaf sample was soaked in water for 4 hours and yet again it was weighed. Then, the same leaf sample was kept in the hot plate at 85 $^{\circ}$C and another time its dry mass was weighed. RWC of leaves was calculated (Aliabadi Farahani *et al.*, 2008). We observed that RWC of leaves in mycorrhiza treated plants was superior to untreated plants. The result in table 8 shows the relative water content of leaves.

Table 7: Effect of inoculation and abiotic stress on relative leaf water content.

S.NO	Treatment	Leaf fresh weight	Turgid weight	Leaf dry weight	RWC
1	Control	0.073	0.183	0.062	9.0
2	Plant at pH 9	0.054	0.227	0.027	18.50
3	Plant at pH 10	0.014	0.091	0.006	9.410
4	Cadmium	0.048	0.121	0.040	9.880
5	Chromium	0.025	0.084	0.012	18.06
6	Both Cd and Cr	0.073	0.208	0.059	9.400

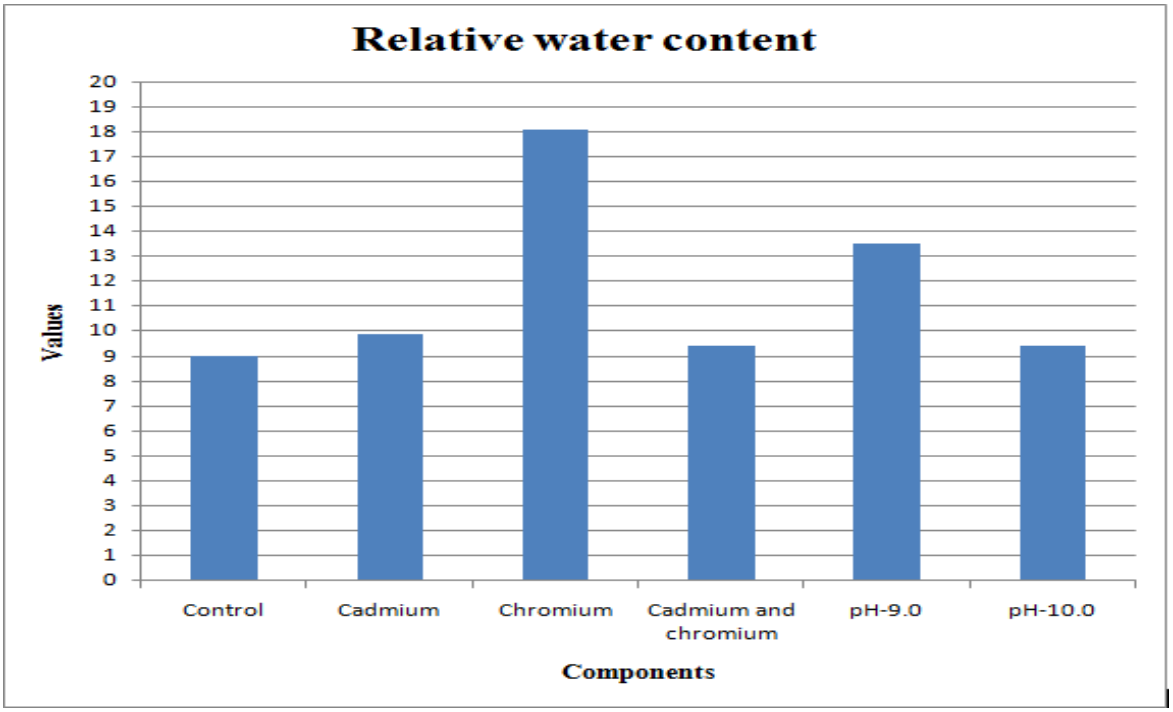


Fig. 7: Total water content of leaves in both mycorrhized plants and non-mycorrhized plants.

Table 8: The result of one way ANOVA on effect of inoculation and abiotic stress on relative leaf water content.

ANOVA: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
0.073	7	0.548833	0.078405	0.008833		
0.183	7	1.797333	0.256762	0.086981		
0.062	7	0.384333	0.054905	0.004751		
ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.17059	2	0.085295	2.54449	0.106368	3.554557
Within Groups	0.603385	18	0.033521			
Total	0.773974	20				

3.5 Effect of inoculation and abiotic stress on photosynthetic pigment

There is no considerable enhancement in photosynthetic pigment range (chlorophylls, xanthophylls, and carotenoid) while the plants undertook stress condition, but mycorrhizal inoculum with PGPB made a huge activist effect on the stressed plants. Under stress, conditions observed augmentation in Chl a, Chl b, xanthophylls and carotenoid owing to pooled application of AMF and PGPB. The total photosynthetic pigments are greater than before while the concentration of arbuscules increased. Chlorophyll increased even as the concentration of chlorophyll b increased. Carotenoid pigments increased as well as xanthophylls content increased when the concentration of whole photosynthetic pigments was increased. A negative correlation between carotenoids and xanthophylls was recorded. We observed the positive correlation of chlorophyll content whereas the negative correlation of both xanthophylls and carotenoids. These results indicated that the harmful effect of both salinity and heavy metal dealing on total pigments was at the severe abiotic stress of the plants. Abiotic stress (salinity and heavy metals) could lead to inferior photosynthesis and efficiency. Diminution in photosynthetic pigment is the major impact of oxidative stress. The result in table 9 shown that photosynthetic pigments of both treated and untreated plants.

Table 9: Effect of inoculation and abiotic stress on Photosynthetic pigment.

S.NO	Treatment	Chl a	Chl b	Chl total	Xanthophylls and carotenoids
1	Control	0.536	0.473	0.762	-0.893
2	Plant at pH 9	0.676	3.317	2.172	-0.834
3	Plant at pH 10	0.463	2.587	2.460	-0.875
4	Cadmiun (Cd)	0.891	0.262	1.083	-0.466
5	Chromium (Cr)	0.395	1.117	2.301	-0.639
6	Both Cd and Cr	0.533	1.188	2.402	-0.838

Table 10: The result of one way ANOVA on the effect of inoculation and abiotic stress on Photosynthetic pigment.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
a	6	4.824	0.804	0.430944
b	6	15.74775	2.624625	5.517658544
c	6	10.42875	1.738125	3.684723694
d	6	3.976425	0.6627375	0.585894189
e	6	7.1415	1.19025	1.862076775
f	6	7.39125	1.231875	2.105572294

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.48839	5	3.097677804	1.310089363	0.286248	2.533555
Within Groups	70.93435	30	2.364478249			
Total	86.42274	35				

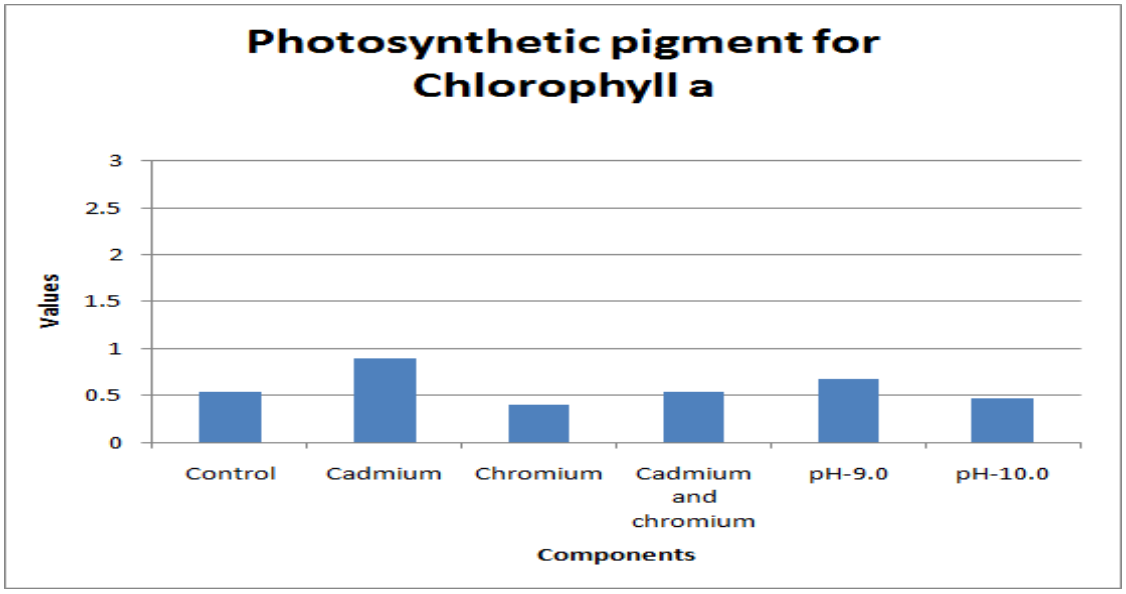


Fig. 8: The presence of chlorophyll a in both mycorrhized and non-mycorrhized plants.

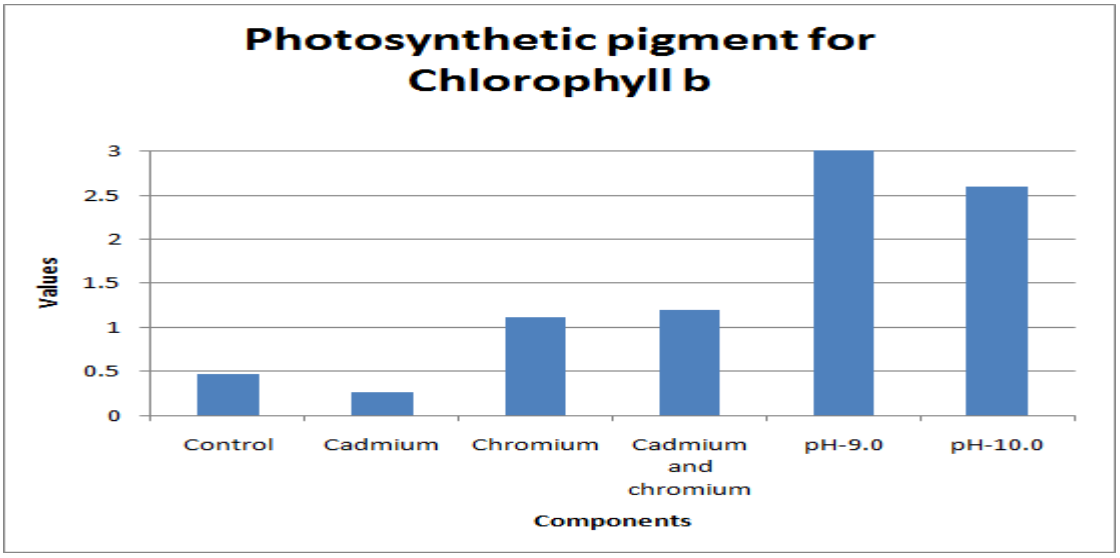


Fig. 9: The presence of chlorophyll b in both mycorrhized and non-mycorrhized plants.

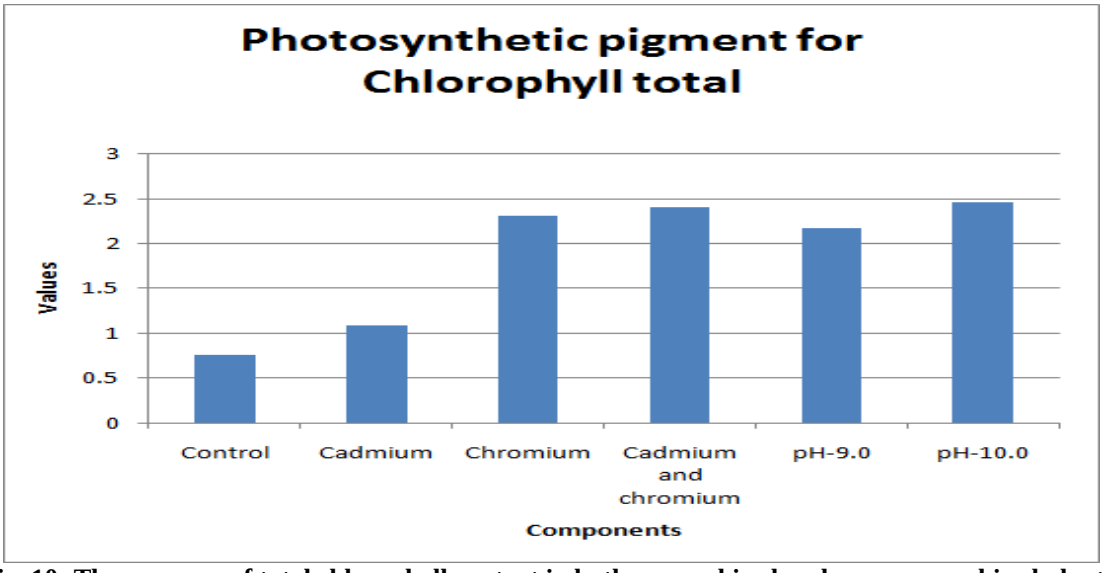


Fig. 10: The presence of total chlorophyll content in both mycorrhized and non- mycorrhized plants.

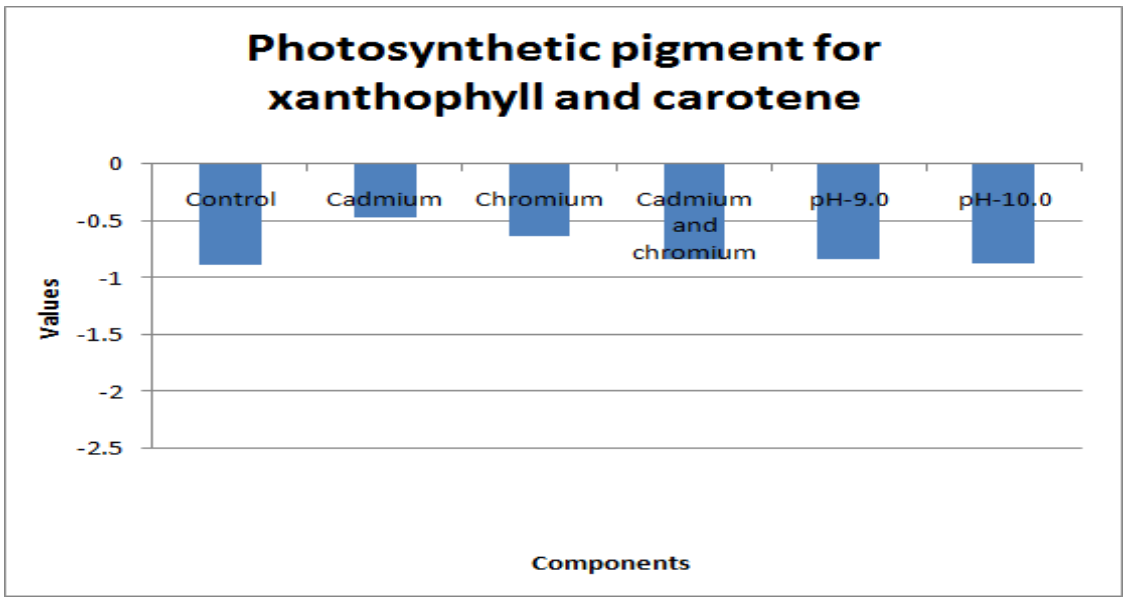


Fig. 11: The presence of total xanthophylls and carotenoid in both treated and untreated plants.

3.6 Effect of inoculation and abiotic stress on hydrogen peroxide content

Fresh tissue of mycorrhized plant was collected and it was homogenized in trichloroacetic acid followed by centrifugation at 12,000 g for 15 min. The supernatant (0.5 ml) was removed from the centrifuge tube and it was mixed with an equal volume of potassium phosphate buffer (pH 7.0) and potassium iodide. Samples were vortexed, and the absorbance was read at 390 nm to quantify the hydrogen peroxide (Abeer Hashem *et al*., 2019). We observed that a higher quantity of hydrogen peroxide present in the non- mycorrhized plants than the control treatment.

Table 11: Effect of inoculation and abiotic stress on hydrogen peroxide content.

S.NO	Treatment	Quantity of hydrogen peroxide
1.	Control	0.027
2.	Plant at pH 9	0.041
3.	Plant at pH 10	0.046
4.	Cadmium	0.043
5.	Chromium	0.016
6.	Both Cd and Cr	0.05

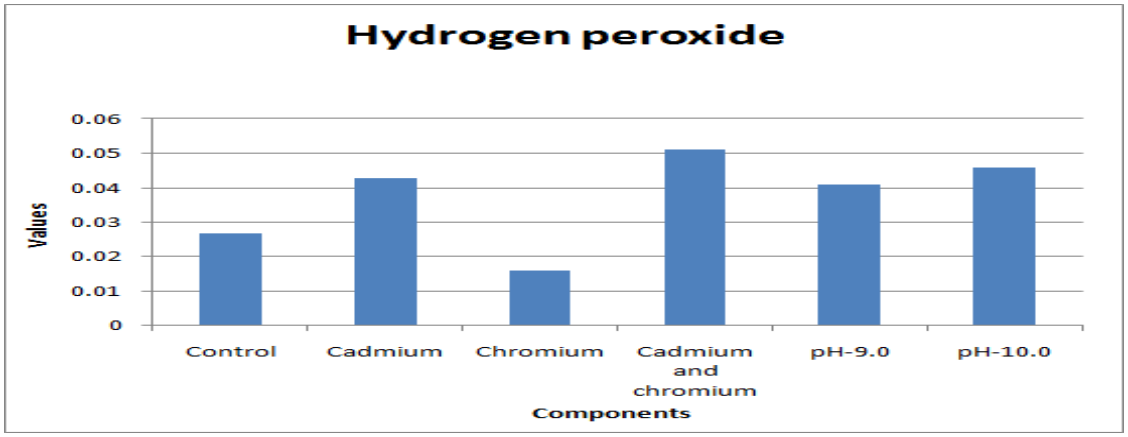


Fig. 12: The amount of hydrogen peroxide present in the mycorrhized and non- mycorrhized plants.

4. Expected result

4.1 Ions estimation

Here we want to describe the kind of results that will be expected by us according to problems identified and methodology proposed. Nutrients uptake will be increased that can be expected due to combining the application of AMF and PGPB. Several studies reported that AMF inoculum can increase the nutrients uptake by plants. Plant metabolism will be increased while incrementing in nutrients uptake. For example, potassium has a capacity for increasing leaf water status. We got high relative water content (RWC) in mycorrhized plants so that increment in nutrients will be expected in AMF treated plants.

4.2 Determination of biochemical parameters

We expect higher SOD, CAT, POD activity in mycorrhizal plants. Because many studies indicated that these enzymes can act against oxidative stress which means enhancement of the oxygen level leading to a reduction in plant growth. We got a higher improvement in morphological traits like a higher length of root and shoot. As the improvement of morphological parameters higher SOD, CAT, POD activity will be expected in mycorrhized plants.

5. DISCUSSION

Most of the plants are affected by the abiotic stresses leading to the death of plant. Abiotic stresses are created from the surrounding environment of plants. Nevertheless, many studies on AM symbiosis have confirmed the contribution of the involved fungus in helping the plant to resist abiotic stress such as drought, salinity, and heavy metal contamination through the implementation of various mechanisms due to fungal symbiosis (Rodriguez *et al*., 2008; Ahanger *et al*., 2014; Salam *et al*., 2017).

Abeer Hashem *et al*., 2018 showed that to overcome the deleterious effect of salinity on growth, a series of tolerance mechanisms are initiated to maintain the growth and development of plants. The upregulation of the antioxidant system, greater accumulation of compatible osmolytes and the efficient compartmentalization of excessive toxic ions into the vacuole are considered important tolerance strategies. The antioxidant defense system comprises both enzymatic and non-enzymatic components, which protect plants from salinity stress by eliminating excess accumulated ROS. Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione reductase (GR), which are intricate electron donors during the enzymatic

neutralization of ROS, while the non-enzymatic antioxidants include ascorbic acid (Vitamin C) and reduced glutathione (GSH), which are involved in stress tolerance. The greater synthesis and accumulation of compatible osmolytes like free proline, glycine betaine, soluble sugars and amino acids under stressed conditions stimulate osmoregulation to maintain the cellular tissue water content, thereby helping plants to maintain growth. Plants exposed to salt stress usually take up ions like sodium, chloride, and potassium from the growth media, and the excess sodium is compartmentalized into the vacuole, transported by the apoplast pathway or excluded from the tissues. Farhana Alam Ripa *et al*., 2019 stated that the plant growth promoting bacteria give rise to enzymes like ACCD, urease, catalase, etc., phosphate solubilization, siderophore and IAA formation and antagonism to phytopathogens which stimulate the plant growth.

Mayada Sabra *et al*., 2018 described that use of various proteomics techniques such as 2D gel electrophoresis, Matrix assisted laser desorption ionization Time of flight (MALDI-TOF), Liquid chromatography spectrometry (LC-MS) have led to the discovery target proteins that place a role in heavy metal detoxification in several plants. Various amino acids, amines, organic acids, phenols, glutathione are some metabolites that are also involved in heavy metal stress tolerance. Improvement of antioxidant response leading to cadmium stress tolerance.

Arbuscular mycorrhizal fungi (AMF) facilitate host plants to grow vigorously under stressful conditions by mediating a series of complex communication events between the plant and the fungus leading to enhanced photosynthetic rate and other gas exchange-related traits (Birhane *et al*., 2012), as well as increased water uptake. Junqin Li *et al*., 2019 reported that the AMF is one of the important group of microbes which can create a symbiotic association with the root of the plants. It can increase the growth of the plants by stimulating the supply of nutrients and water uptake to reduce the abiotic stresses, such as salinity and drought. Farhana Alam Ripa *et al*., 2019 stated that the AMF improves plant growth through the production of some significant enzymes like 1- Aminocyclopropane-1-carboxylic acid deaminase (ACCD), urease, catalase, etc., phosphate solubilization, siderophore, and Indole-3-acetic acid (IAA) formation. Abeer Hashem *et al*., 2018 estimated an plant growth-promoting factors like nutrients, growth hormones and antioxidant enzymes to report the plant growth condition.

Bo Meng *et al*., 2019 stated that the AM fungal hyphae can explore soil pores that the root hair cannot contact, accessing water and nutrient sources that are unavailable to non-inoculated plants. AMF can make better plant performance, change the plant–water relationship, and increase plant productivity under abiotic stress. AMF can increase water use efficiency (WUE) by improving stomatal conductance and increase antioxidant enzyme activity to reduce peroxidative damage. Eslam Abdel-Salam *et al*., 2018 described that most of the plant families form a mutualistic relationship with the *Arbuscular mycorrhizal* fungi. The fungus associates with the plant roots in such a relationship enhance the plant's ability to absorb water and nutrition via increasing the absorbing area through its large surface area of mycelium. The plant, in turn, provides the fungus directly with needed carbohydrates including glucose and sucrose. This symbiotic relationship between plants and the *Arbuscular mycorrhizal* fungi (AMF) is a key factor helping plants to resist abiotic stress.

Sandhya Vardharajula *et al*., 2016 reported that plant growth- promoting bacteria (PGPB) determine resistance to water stress in plants others than the original isolation. A set of abiotic stresses were able to perform different plant growth-promoting (PGP) activities and higher root colonization suggesting that the halophilic/halotolerant bacteria inhabiting salty and arid ecosystems have a potential to promote plant growth under salinity and drought condition. Microbial communities below the ground level influence the selection on plant traits by mitigating the effects of abiotic stress on plant populations.

Pseudomonas fluorescens has the potential agents for the biocontrol which restrain plant diseases by defending the seeds and roots from fungal infection. This effect is the result of the production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide (O'Sullivan and O'Gara, 1992).

6. CONCLUSION

We concluded that abiotic stresses like heavy metals and salinity shaped pessimistic impacts on the plants. We step forward to reduce these harmful impacts using naturally occurring microorganisms like AMF and PGPB. Impacts of abiotic stress could be mitigated by combining the application of AMF and PGPB. The results of our study showed the improvement of physical, biochemical and morphological parameters due to the inoculation of both AMF and PGPB. Our expected results suggest that amalgamation of AMF and PGPB also can improve the nutrients uptake and antioxidant enzyme activity.

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