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Original Research Article

Effect of Enzymes, Biotic, Abiotic Stresses and Metal Toxicity in *Brassica napus*

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Abstract

Canola is an important crop with low levels of fatty acid and genetically produced from rapeseed. Canola oil is obtained by extract sources. Heavy metals are a unique form of toxin because they may exist in the environment for a longer period. Exogenous administration of gibberellic acid has been proven in several studies to increase crop output by affecting an important physiological process. This study of the aimed and objectives included the nickel sulphate stress in quantity 300 µM and gibberellic acid in quantity 0.25 mM in specific quantity and their effects on different plant functions. Super canola under stress condition when 0.25 mM GA3 was applied. Whereas, minimum reduction was observed in cv. Sherilla under stress condition when 0 mM GA3 was applied. Maximum Catalase was observed in cv. Results showed the Peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) are the most important protective enzyme systems in plants, and also the most important reactive oxygen species clearance system. Maximum Total Amino acid was observed in cv. Sherilla under control condition when 0 mM GA3 was applied. However, effect of GA3 application was observed non-significant and same for as varietal difference among two genotypes was observed non-significant. Overall, super canola performed better under stress or non-stress condition. Mustard plants exposed to nickel showed decreased morphological characteristics, which might be related to the degradation of plant architecture, which limits the capacity of water content and minerals absorption by plants, resulting in total development of plant loss.

Keywords: Peroxidase, catalase, superoxide dismutase, reactive oxygen species.

INTRODUCTION

Canola is an oleaginous crop with low levels of fatty acid (EA) that was genetically produced from rapeseed. Canola is most world leading plant, from which oil is obtained. Almost 16% of vegetable oil seed is produced from it and worldwide third most oil seed plant after palm and soyabean productivity. It has generally recognized that oil of canola is obtained by rapeseed, for example rapeseed oil (>5%), rapeseed canola equal oil low EA oil rapeseed [1, 2]. Due to its magnificent qualities, for example its extraordinary production and resistance to many diseases, this crop has most economically significant value. Among the 37 species of the Brassicaceae family, the Brassica genus contains *B. napus* widely grown worldwide. *Brassica napus* is mostly grown for the production of oil. The production of oil seed is huge but remains inadequate to complete with the daily necessity of the people [2, 3]. Economically, this inadequate yield ascribes to different stress i.e., biotic and abiotic. Among all the stress, salinity stress considered as one of the major limitations that restrict the yield of crop by lowering the photosynthetic rate, many metabolic process and synthesis of protein. The eatable oil is obtained from mustard seeds (*Brassica juncea* L.) that have properties like oil of canola. Vegetable oil is also obtained from these seeds of canola. Whereas the seeds of mustard are

not involved in oil production and because of their pungent smell, they are principally used in the trade of spice as a condiments source. Furthermore, the seeds of brown mustard can be used in different medicines. They are used as animal fodder with high nutrition value as well as eatable oil is also obtained from them [3, 4].

Heavy metals have high density and mostly toxic in nature for human, plants, and animals regardless of their concentrations and have high atomic density five times greater than water or more than 4 g/cm3 or more than 5 g/cm3. Heavy metals are a unique form of toxin because they may exist in the environment for a longer period. Since they can not be broken down into non hazardous forms [5, 6]. After the industaril revolution heavey metals are increasing drastically which is a genuine warning to mortal health and the circumstances. Heavy metals launch toward the surrounding by natural and evolution derivation. Among the natural origins the most remarkable sources are erosion, volcanic eruptions, eroding of minerals while extraction and processing of minerals, industrial activities and addition of phosphatic fertilizers are the phylogeny history of heavy metal pollution in our agro ecosystem [7, 8].

Substantial testing on mammals, human beings and certain plants has demonstrated the toxicity of nickel as an environmental contaminant. Because of the characteristics of the soil, the environmental mobility of Ni^{+2} varies. Nickel contamination also poses a risk to the safety of ground water. Accumulation of Ni in soil can lower the amount of S, Mn, Ng, Fe, Zn and Cu from plant. Higher amount of Ni^{+2} levels cause symptoms associated with chlorosis, necrosis, and toxicity. The mineral nutritional balance and functions of the cell membrane of plants growing in soils containing high Ni^{+2} concentrations are disturbed. Ni^{+2} disturbs the H-ATPase activity of the lipid structure and cell membrane. It is also established that high Ni^{+2} levels inhibit plant water intake [9, 10]. Excess Ni levels in sensitive plants' tissues can result in hazardous symptoms including stunted growth, lower yield, photosynthetic inhibition owing to lower chlorophyll content, and stomatal closure. Plant species, developmental stage, Ni exposure concentration and duration, and culture conditions are all factors that impact Ni phytotoxicity. Several heavy metals have been shown to cause a stress-induced morphogenic response in the root system, which includes main root shortening and lateral root induction, even at low concentrations. Auxin, cytokinin, and ethylene phytohormone metabolism, as well as changes in reactive oxygen species (ROS) and nitric oxide (NO) signaling, are thought to be responsible for these growth changes [11, 12].

The purpose of this research was the effect of effect of enzymes, biotic, abiotic stress and metal toxicity in *Brassica napus*.

MATERIAL AND METHODS

The field work undertaken to investigate the exogenous influence of gibberellic acid on two *Brassica napus* L. canola crop varieties organism under threat from nickel. After about 30 days of seedling arrival, the stress of nickel sulphate was applied to both varieties, and the next level of stress was applied the one week of first stress application.

Enzyme extraction:

Enzymatic antioxidants of mustard plant remained purified by crushing 0.25g of fresh leaf material in 5 ml of 50 mM cooled potassium phosphate buffer (pH 7.8). This standardized substantial remain centrifuged for 15 minutes at 15000 rpm at 4ºC. The pills remain castoff and residual remain castoff for determination of events of dissimilar enzymatic antioxidant.

Superoxide dismutase (SOD)

The activity of superoxide dismutase was estimated. Nitroblue tetrazolium (NBT) was used to make sure about the activity of SOD. The response reagent was made out of 100 µl phosphate buffer, 5ml refined water, 50 µl NBT, 100 µl methionine and 50 µl of protein extract put in cuvette and were held under light for 15 min. the reading was estimated under 560 nano meter (nm) with a clear reading of 250 µl of phosphate buffer [11, 12].

Peroxidase (POD):

The POD solution (3ml) comprised of 750 mM phosphate buffer, 100 mM Guaiacol, 100 mM, 50 µl enzyme extract. The reaction remain started by addition of enzyme extract. Fluctuations in absorbance of solution at 470 nm remain studied at 0, 30, 60 and 90s. Absorbance change of 0.01 units one minute and 30 seconds was based on one unit of POD [11, 12].

Catalase (CAT):

The catalase reaction solution 3ml confined to 1.9 ml phosphate buffer (PH 7.8), 1 ml H2O2 and 100 µl enzyme extract. The reaction remain originated by addition of enzyme purified. Fluctuation in absorbance of reaction at 240 nm remain study at 0, 30, 60, 90s. absorbance change of 0.01 units one minute and 30 seconds was based on one unit of catalase [11, 12].

Malondialdehyde (MDA)

Chromium convinced oxidative injury to membranes remain assessed by measuring the quantity of malondialdehyde in tissues. 0.5 g of fresh leaf substantial remained standardized in 3 ml of 5% (w/v) TCA (trichloro acetic) at 4ºC. Then the homogenate was centrifuged at 1200 rpm 15 minutes. Take 1ml supernatant, 3ml of 0.5% (v/v) thiobarbituric acid (prepared in 20% TCA) was added. The blend remain kept at 95ºC in a water bath and place in ice bath. When temperature of sample remain 25 ºC, they remained centrifuged for 10 minute at 1000 rpm. In conclusion, the absorbance of combination remain studied at 532 nm.

The non-selected concentration was taken at 600 nm was deducted from all values.

Hydrogen peroxide determination

The mixture remain centrifuged for 20 minutes at 12000 rpm. The one ml of potassium iodide and 0.5 ml potassium phosphate buffer (Ph 7.8) remain supplemented to 0.5 ml of the enzyme extract. This blend remain vortexed cautiously and its absorbance read at 390 nm.

Total soluble proteins:

The soluble proteins of the sample were determined by Bradford method. To extract protein, 0.5 g fresh leaves remain crushed by a tissue grinders in 5 ml of 50 mM ventilated phosphate buffer (pH 7.8) placed in ice bath. The homogenous remain centrifuged at 15000 rpm for 15 min at 4ºC. The supernatant was used for protein determination. 0.1 ml was taken in each Eppendorf tube and assorted by 5 ml of Bradford reagent. This sample remained incubated at 37 ºC for 10 to 15 minutes laterally with blank and absorbance was noted at 595 nm.

Free amino acids:

The free amino acid of sample was determined. Took 0.5 ml of extract in 25 ml test tubes, add 0.5 ml of 10% pyridine and 0.5 ml of 2% ninhydrin solution in test tubes. Heated tubes in water bath for about 30 minutes at 100 ºC. made the volume of test tubes to 25 ml with distilled water and took reading at 570 nm.

Statistical Analysis

Information was dissected statistically by ANOVA under CRD in three replications. LSD test at likelihood level 0.05% was utilized for looking at treatment indication.

RESULTS AND DICUSSIONS

SOD (mg/g FWT)

Application of metal stress caused significant ($P \leq 0.05$) reduction in SOD of both genotypes. Maximum reduction SOD was observed in cv. Super canola under stress condition when 0 mM GA3 was applied. Whereas, minimum reduction was observed in cv. Super canola under stress condition when 0.25 mM GA3 was applied. Maximum SOD was observed in cv. Sherilla under control condition when 0.25 mM GA3 was applied. However, effect of GA3 application was observed significant. While the variety difference among two genotypes was observed non-significant. Overall, sherilla performed better under control and stress condition.

POD (mg/g FWT)

Application of metal stress caused significant ($P \leq 0.05$) reduction in POD of both genotypes. Maximum reduction POD was observed in cv. Super canola under stress condition when 0 mM GA3 was applied. Whereas, minimum reduction was observed in cv. Sherilla under stress condition when 0 mM GA3 was applied. Maximum POD was observed in cv. Sherilla under control condition when 0.25 mM GA3 was applied. However, effect of GA3 application was observed non-significant. While varietal difference between two genotypes was observed significant. Overall, sherilla performed better under control and stress condition.

Catalase (mg/g FWT)

Application of metal stress caused non-significant reduction in Catalase of both genotypes. Maximum reduction Catalase was observed in cv. Super canola under stress condition when 0.25 mM GA3 was applied. Whereas, minimum reduction was observed in cv. Sherilla under stress condition when 0 mM GA3 was applied. Maximum Catalase was observed in cv. Super canola under control condition when 0.25 mM GA3 was applied. However, effect of GA3 application was observed non-significant and same for as varietal difference among two genotypes was observed significant. Overall, super canola performed better under control and stress condition.

MDA (nmol/ml)

Application of metal stress caused non-significant reduction in MDA of both genotypes. Maximum increase MDA was observed in cv. Sherilla under stress condition when 0 mM GA3 was applied. Whereas, minimum increase was observed in cv. Super canola under stress condition when 0 mM GA3 was applied. Maximum MDA was observed in cv. sherilla under control condition when 0.25 mM GA3 was applied. However, effect of GA3 application was observed non-significant and same for as varietal difference among two genotypes was observed non-significant. Overall, sherilla performed better under control and stress condition.

H_2O_2 (µmol/g FWT)

Application of metal stress caused non-significant reduction in H_2O_2 of both genotypes. Maximum increase H_2O_2 was observed in cv. Sherilla under stress condition when 0 mM GA3 was applied. Whereas, minimum reduction was observed in cv. Super canola under stress condition when 0.25 mM GA3 was applied. Maximum H_2O_2 was observed in cv. Sherilla under control condition when 0 mM GA3 was applied. However, effect of GA3 application was observed non-significant and same for as varietal difference among two genotypes was observed non-significant. Overall, Sherilla performed better under control and stress condition.

Total soluble protein (mg/g FWT)

Application of metal stress caused non-significant reduction in Total soluble protein of both genotypes. Maximum reduction Total soluble protein was observed in cv. Super canola under stress condition when 0.25 mM GA3 was applied. Whereas, minimum reduction was observed in cv. Sherilla under stress condition when 0.25 mM GA3 was applied. Maximum

Total soluble protein was observed in cv. Super canola under control condition when 0.25 mM GA3 was applied. However, effect of GA3 application was observed non-significant. While varietal difference among two genotypes was observed significant. Overall, super canola performed better under control and stress condition.

Total Amino acid (mg/g FWT)

Application of metal stress caused non-significant reduction in Total Amino acid of both genotypes. Maximum reduction Total Amino acid was observed in cv. Super canola under stress condition when 0.25 mM GA3 was applied. Whereas, minimum reduction was observed in cv. Super canola under stress condition when 0 mM GA3 was applied.

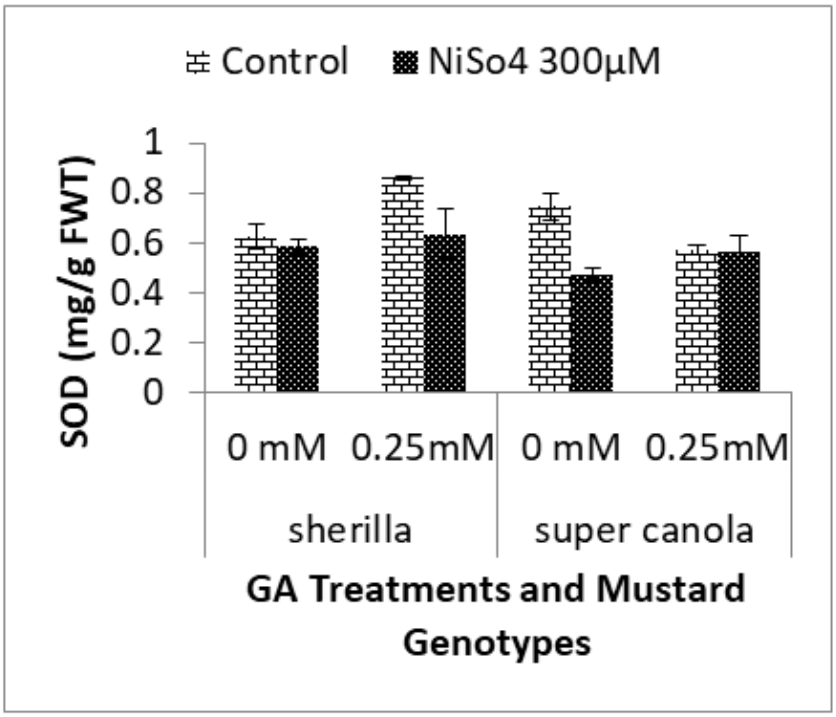

Fig-1: Effects of the SOD of on Mustard (*Brassica napus* L.) genotypes

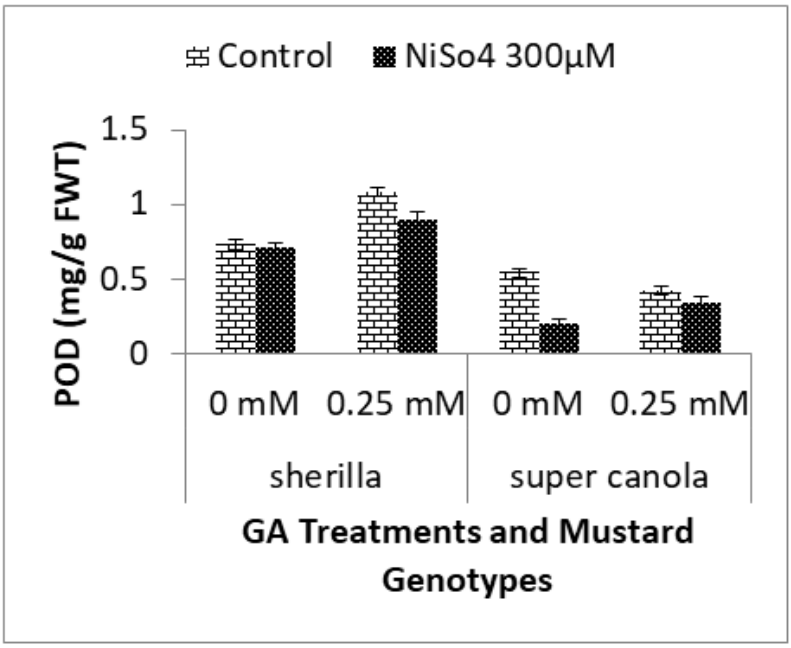

Fig-2: Effects of the POD of on Mustard (*Brassica napus* L.) genotypes

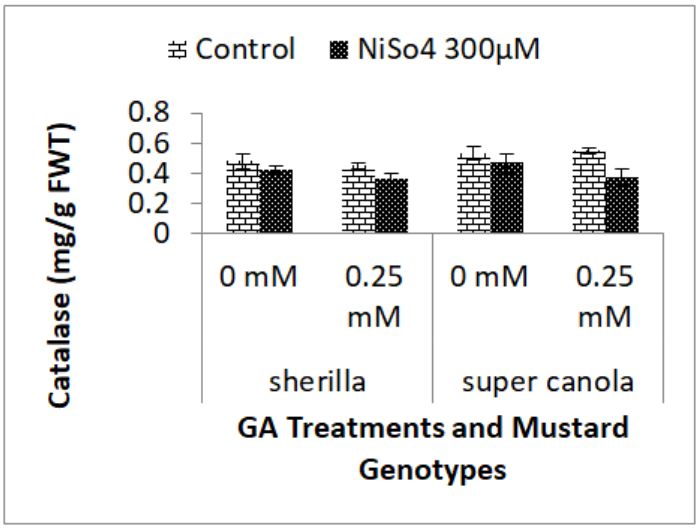

Fig-3: Effects of the catalase on Mustard (*Brassica napus* L.) genotypes

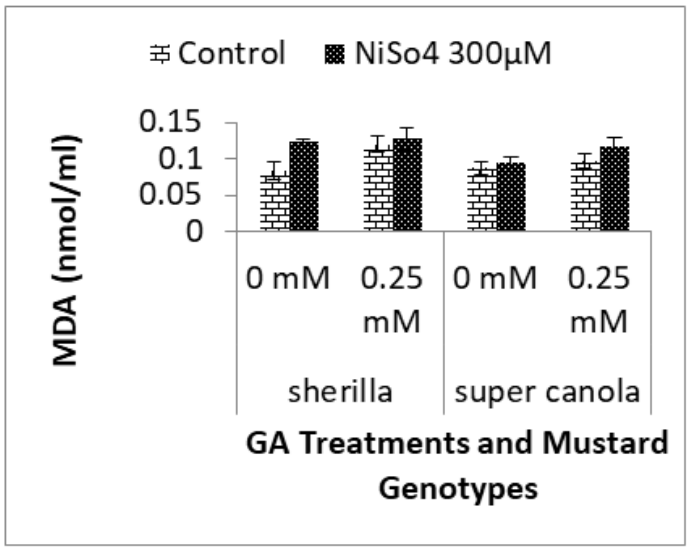

Fig-4: Effects of the MDA on Mustard (*Brassica napus* L.) genotypes

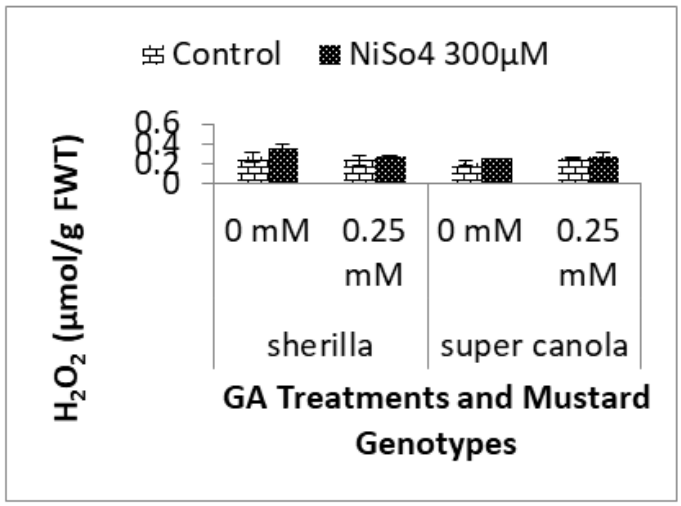

Fig-5: Effects of the hydrogen peroxide on Mustard (*Brassica napus* L.) genotypes

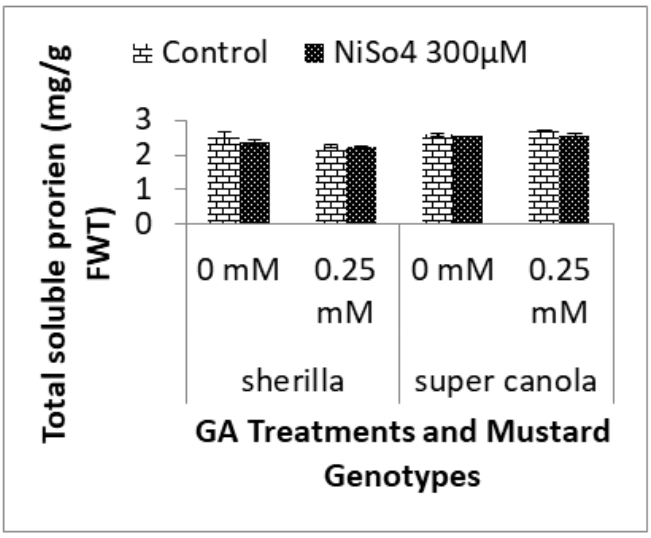

Fig-6: Effects of the total proteins on Mustard (*Brassica napus* L.) genotypes

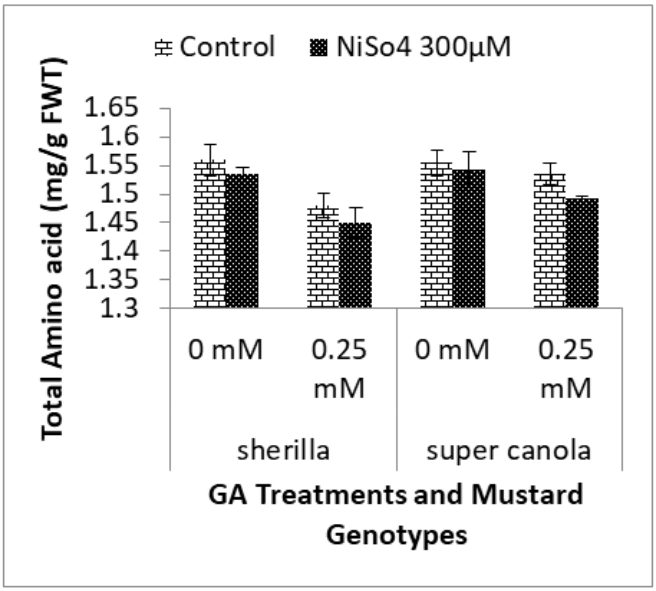

Fig-7: Effects of the total amino acids on Mustard (*Brassica napus* L.) genotypes

Oilseed rape is uniformly a cheaply valuable plant for oil production Owing to its rapid development, high biomass yield and heavy metal assimilation, oilseed rape has been extensively deliberated for its impending phyto-extraction, which largely depends on the efficacy of metal uptake and impending transition, which depends largely on the efficacy of metal concentration and transferred within the different plants components [13, 14]. Ni is said to have a detrimental influence on development and germination of plants. At greater concentrations, Ni becomes poisonous to plants and has a negative impact on plant development and metabolism. Seed germination is inhibited when Plants are subjected to elevated nickel levels. Growth inhibition in canola plants might be due to Ni-induced changes in key metabolic processes, such as photosynthesis and photo assimilates transport from leaves. Mustard plants exposed to nickel showed decreased morphological characteristics, which might be related to the degradation of plant architecture, which limits the capacity of water content and minerals absorption by plants, resulting in total development of plant loss. The present investigation shows result that non-significant salt stress cause reduction in carotenoids. These findings matched those of earlier research. The reason behind the decrease in carotenoids might be that it is capable of destroying leaf stomata. The outcomes of the current research are as follows: significant salt stress cause reduction in SOD, POD. The findings matched those of previous research. Abrupt decrease at a greater level, Severe oxidative damage might cause Ni stress [15, 16].

The present investigation result showed that non-significant salt stress cause reduction in total soluble protein. These findings were in line with those of. The present investigation result showed that non-significant

salt stress cause reduction in total amino acid. These findings matched those of the previous study also protein content was observed to be lower in nickel treated seedlings, presumably due to a reduction in amino acid metabolism. The current study found that severe salt stress causes a non-significant rise in enzymatic antioxidant parameters such as H2O2 and MDA in mustard plants. These results were in accordance with the findings of, which rises in mustard Ni-treated plants and results of malondialdehyde content decreased that showed resemblances with the work [17-19].

CONCLUSION

The anti-oxidative enzymes like as SOD, POD, catalase was decrease under nickel stress. While, greatly improve under foliar application of gibberellic acid. MDA and H2O2 increases under foliar application of gibberellic acid on the other hand Under salt strain, total amino acids and total soluble protein decreased. Higher amount of Ni^{+2} levels cause symptoms associated with chlorosis, necrosis, and toxicity. The mineral nutritional balance and functions of the cell membrane of plants growing in soils containing high Ni^{+2} concentrations are disturbed.

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