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METHYL JASMONATE REGULATED ANTIOXIDANTS AND OXIDATIVE STRESS MANAGEMENT IN *CAJANUS CAJAN* (L.) MILLSP. UNDER COPPER STRESS CONDITION

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ABSTRACT

In the present study, dynamic potential of Methyl-jasmonate (Me-JA) in seedlings of Cajanus cajan (L.) Millsp. was explored under copper stress. Methyl jasmonate is a plant growth regulator which has been studied to shield the plant from various types of stresses. Our study first time reports the defensive effect of Me-JA under copper stress in C. cajan seedlings. Me-JA declined lipid peroxidation significantly which was upto sophisticated level in seedlings grown in Cu stress alone without Me-JA treatment. Copper stress increased the antioxidant potential which is also increased in seedlings supplemented with Me-JA. Activities of antioxidant enzymes such as SOD, POD and CAT were increased in Me-JA pre-sowing soaking treatments as well as under Cu stress. However, in Me-JA treated seedling grown under Cu stress activities of SOD, POD and CAT showed less increase. Non-enzymatic antioxidants like Vitamin A, C and E also increased in Me-JA treated seedlings with or without Cu stress. However, decrease in root length was seen in Me-JA untreated seedlings grown in Cu stress as compared to the control seedlings grown in distilled water while Me-JA treated seedlings grown in Cu stress condition show increased root length. An increase in shoot length was observed in Me-JA treated seedlings grown in Cu stress as untreated seedlings.

Keywords: Antioxidants, Lipid Peroxidation, Methyl Jasmonate, Oxidative Stress, Pigeonpea

I. INTRODUCTION

Copper (Cu) is an indispensable micronutrient for plant growth and development. It is a core ion in plant pigments and enzymes such as plastocyanin, polyphenol oxidase, superoxide dismutase, tyrosinase, cytochrome *c* oxidase [1]. It plays an important role in various physiological processes such as cell wall metabolism, hormone signaling, photosynthesis and respiration [2-3]. However, at high concentration, Cu causes oxidative damage to the plant cell which affects plant growth and development. Increased concentration of Cu in soil is responsible for hyper accumulation by plants leads to induction of oxidative stress in *Oryza sativa* [4] and also caused programmed cell death in *Tigrious japonicas* plants [5]. In the seedlings, grown under Cu stress conditions, inhibition of seed germination, root length and shoot length was reported in tomato, wheat and barley [6-8]. Oxidative stress in plants leads to accumulation of reactive oxygen species (ROS). The ROS are highly reactive and toxic radicals which comprise of $O_2^{\bullet}$, $H_2O_2^{\bullet}$, $^1O_2^{\bullet}$, HO^2, $OH^{\bullet}$, $ROOH^{\bullet}$, $ROO^{\bullet}$ and $RO^{\bullet}$ and causes damage to carbohydrates, proteins, lipids, DNA which ultimately results in cell death. To balance these

ROS enzymatic antioxidant systems involving scavengers, such as SOD, POD, APX and CAT and non-enzymatic such as ASH, GSH, a-tocopherol, carotenoids and flavonoids [9]. Pigeon pea (*Cajanus cajan* (L.) Millsp.) is sixth major pulse crop grown tropical and subtropical regions all over the world. The Indian subcontinent accounts for about 92% of the global production of *C. cajan*. Due to highly rich protein content (21%), it is one of the nutritious diet components in various parts of the world. It contains various medicinal and toxicological properties such as antimicrobial activities, hepato-protective activity, antisickling, hypo-lipidemic, antioxidant effects, antidiabetic activities.

Jasmonate (JA's) are oxylipin signaling molecules synthesized through octadecanoid pathway from fatty acid named Linolenic Acid which is released by the disintegration of plasma membrane. JA's are central regulators in many processes involved in growth and development *viz.* germination, root/shoot growth, tuber formation, tendril coiling, stomatal closure, senescence and abscission etc. Methyl-jasmonate (Me-JA) was identified as a fragrant molecule in 1962 from the essential oil of *Jasminum grandiflorum* and later on from *Rosmarinus officinalis*, while the free JA was first isolated from the culture filtrate of the fungus *Botryodiploidia theobromae* in 1971 and *Fusarium oxysporum* [10-13]. These are also involved in defense responses against herbivores, nematodes, necrotrophic pathogen and microorganism. They also work as key regulator in alleviating abiotic stresses like salt, osmotic, temperature, UV, heavy metal, ozone [14-16]. The present study was commenced to check the defensive potential of Me-JA seed priming in *C. cajan* seedlings under Cu stress on antioxidants accumulation.

II. MATERIAL AND METHODS

1. Plant Material and Stress Treatments

Pigeonpea (*Cajanus cajan* AL-201) seeds were purchased from Department of Plant Breeding and Genetics, Punjab Agriculture University, Ludhiana, India. Carefully chosen healthy seeds were treated with 5% hypochloride (v/v), a commercial fungicide for 5 minutes. These sterilized seeds were soaked DW as control, for 6 hours and then grown in 0, 1mM, 5mM and 10mM concentrations of Cu in petriplates line with filter paper. Seeds of each treatment were sowed in triplicate and in 10mL of DW or 10mL of Copper sulphate pentahydrate at 24˚C under controlled condition (200 PAR light, 80% humidity and 16±8 photoperiod).15 days after sowing (DAS) seedlings were harvested, frozen in liquid nitrogen and stored at -80°C until used.

2. Lipid Peroxidation and Antioxidant analysis

2.1. Malondialdehyde (MDA) content: The level of lipid peroxidation was measured by estimation of Malondialdehyde (MDA) content using Thiobarbituric acid reaction method of Heath and Packer (1968)[17].

2.2. Superoxide Dismutase: Superoxide dismutase (EC 1.15.1.1.) activity was measured by using the method of Kono (1978) method [18]. To 0.5 ml of enzyme extract, 1.8µl of 50 mM of Sodium Carbonate buffer (pH-10), 750 µl of 96 µM NBT and 150µl Triton X-100 were added. The reaction was initiated by adding 0.4ml of 1mM hydroxylamine hydrochloride. Absorbance was taken at 540nm using spectrophotometer mentioned elsewhere, and activity of SOD was taken as an increase in absorbance for 2 min at 25˚C.

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2.3. Guaiacol peroxidase (EC 1.11.1.7): Guaiacol peroxidase (EC 1.11.1.7) was assayed by mixing 50µl of Guaiacol, 30µl of H_2O_2 and 3ml of potassium phosphate buffer and enzyme extract. Blank was prepared by adding all the reagents except enzyme extract by following the method of Putter (1974) [19].

2.4. Catalase activity (EC1.11.1.6): Catalase activity was assayed by Aebi *et al*., (1984) [20] method by mixing H_2O_2, potassium phosphate buffer and enzymes extract.

2.5. Vitamin A: Vitamin A estimation was done according to Bayfield and Cole (1980) method [21]. Homogenate was mixed with 1.0ml of saponification mixture and refluxed at 60°C in the dark followed by cooling with 20ml water. Vitamin A was extracted with 10ml petroleum ether. TCA reagent (2.0ml) was added rapidly with absorbance at 620nm in a spectrophotometer.

2.6. Vitamin C: Vitamin C was estimated according to the Chinoy *et al*. (1976) method [22]. 2 ml of plant extract was prepared and added 8 ml of 2, 6-dichlorophenol indophenols dye. OD was recorded at 530 nm.

2.7. Vitamin E: Vitamin E was estimated by using Rosenberg (1992) method [23]. Tissue sample was mixed slowly with 0.1 N sulphuric acid and incubated at room temperature for overnight and then filtered. To the 1.5 ml of tissue extract added 1.5ml of xylene and centrifuged. Then 1.0ml of xylene was separated and mixed with 1.0ml of 2, 2-pyridyl and noted the absorbance at 460nm. Then in the beginning with blank added 0.33 ml $FeCl_3$ and mixed well. After 15 minutes read the test and standard against the blank at 520nm.

2.8. Antioxidant potential: The DPPH radical scavenging assay was performed as described by Miliauskas *et al*., (2004) [24] with slight modifications. The kinetics of 400 µl aqueous extract in 2.8 ml of DPPH (80µM in ethanol) were registered in 5 minutes by monitoring DPPH disappearance at 515 nm.

Statistical Analysis

All analysis was done on a completely randomized design. All data obtained was subjected to one way analysis of variance (ANOVA) using Graphpad Prism 5.0 software. Each data was the mean of three replicates (n=3) except for shoot and root length of *C. cajan* L. seedlings (n=5) and comparisons of p-values <0.05 were considered significant and different from control.

III RESULTS AND DISCUSSION

3.1 Effect of Cu and Me-JA treatment on Lipid peroxidation

Malonaldehyde (MDA) is the product of lipid peroxidation of plasma membrane indicating the degeneration of plasma membrane which increases drastically whenever any stress comes to the cell.

Table 1: effect of Me-JA and Cu treatment on enzymatic antioxidants in 15 days old *Cajanus cajan* seedlings

Treatments	MDA content	SOD (Unit Activity $min^{-1}gm^{-1}$protein)	POD (Unit Activity $min^{-1}gm^{-1}$protein)	CAT (Unit Activity $min^{-1}gm^{-1}$protein)
Control	532±6.30	$24±2.00^{c}$	$57.2±3.80^{a}$	$4.22±1.20^{a}$
5mM Cu	$769±4.50^{a}$	$55±3.10^{a}$	$19.08±2.22^{a}$	$11.3±2.30^{a}$
1µM +5mM Cu	$626±4.80^{h}$	$47.5±2.60^{a}$	$347.1±0.89^{a}$	$9.04±0.68^{a}$

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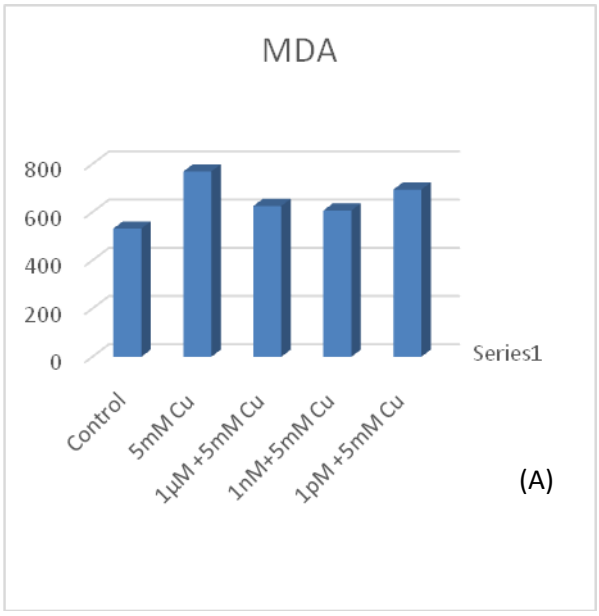
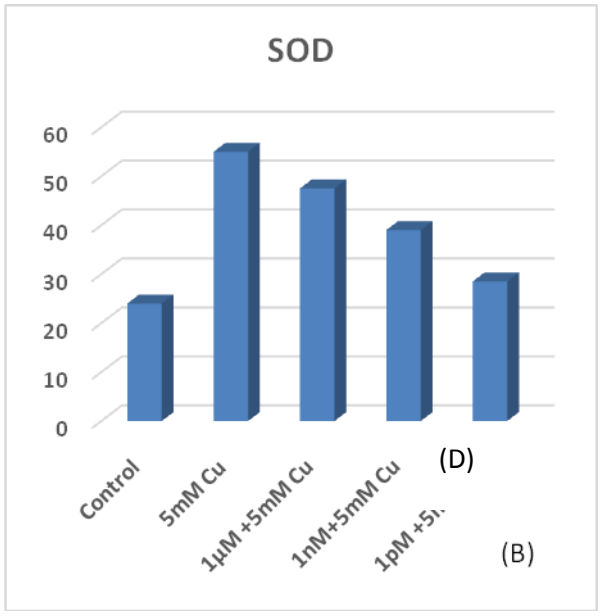
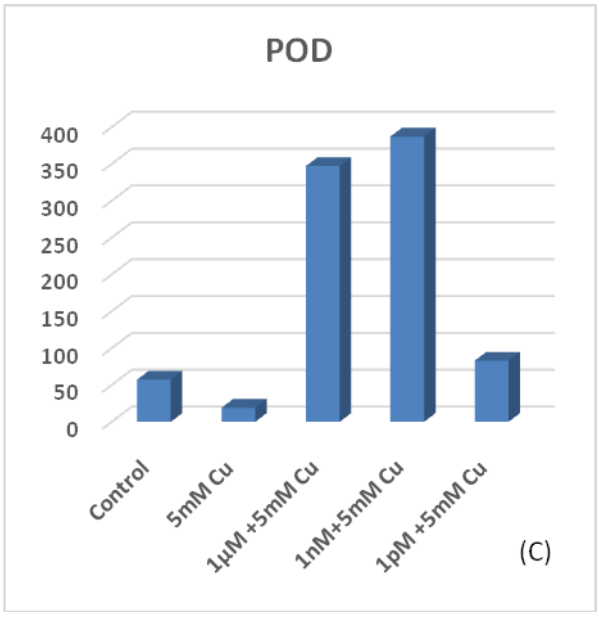
1nM+5mM Cu	607±5.20[h]	39±2.78[b]	387.1±0.58[a]	4.62±0.21[a]
1pM +5mM Cu	694±5.26[d]	28.5±3.50[b]	83.09±0.22[a]	1.52±0.98[a]

MDA content was taken as marker of stress in the present study and the data for *C. cajan* seedlings. MDA content raised from Cu alone or in different combination of Me-JA and Cu is presented in **Fig 1** and **Table 1**. MDA was increased in 5mM Cu to 44 % as compared to the control. However, in Me-JA treated seedling the MDA content was lowered and only 14% increase was seen in 1nM Me-JA treated seedlings grown in 5mM Cu stress. Fidalgo *et al.,* (2013) reported similar increase in MDA content during Cu stress [25]. Thounajam *et al.,* (2012) reported increase in oxidative stress in rice seedlings which was reduced when Me-JA treatment was given to the plants [4].







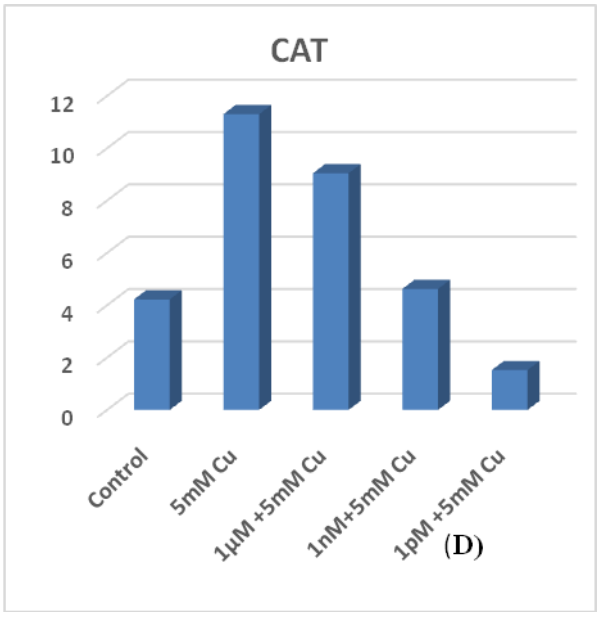


Figure (A-D): effect of Me-JA and Cu treatment on enzymatic antioxidants of 15 days old *Cajanus cajan* seedlings

3.2 Effect of Cu and Me-JA treatment on enzymatic antioxidants

Antioxidants SOD, CAT, GPOD are the few enzymes which act as antioxidants and work on various ROS to mitigate them into other stable products and thus protect the system from oxidative stress. SOD is an enzyme which works on superoxide to convert them into more stable ROS product H_2O_2. The data regarding SOD in *C. cajan* seedlings raised under different treatments of Cu alone and in Me-JA combination is presented in **Fig 2** and **Table 1**. (Umin^{-1}g^{-1} Protein). Activity of SOD was increased to 129% Cu stress while less increase was seen when Me-JA treated seedlings were grown in 5mM Cu as compared to CN DDW seedlings. Our results are in agreement with Aftab *et al.,* [26] where Me-JA treatment induced activity of SOD and CAT.

POD is an enzyme which was also working as peroxidase enzyme to catalyse H_2O_2 into H_2O and O_2. Unmatched results of POD were recorded in *C. cajan* seedlings raised under different treatments of Me-JA and Cu alone or in diverse combinations as shown in **Table 1 and Fig 3**. POD activity was decreased to 67% in Cu stress but increased in Me-JA treatments and highest increase was reported in 1nM Me-JA treated seedlings grown in Cu stress. Zhao *et al.,* (2010) reported decrease in POD activity in *Lolium perene* under Cu stress [27].

CAT is the enzyme which works on end products of SOD and NADPH oxidase activities which is H_2O_2 and catalyse it into H_2O and O_2. Me-JA showed variable results for CAT activity in *C. cajan* seedlings raised with or without Cu stress as shown in **Table 1 and Fig 4**. CAT activity was increased to 167% in Cu stress but decreased in dose dependent manner in Me-JA treated seedlings grown in Cu stress highest upto 64% decrease was seen in 1pM Me-JA treated seedlings grown in Cu.

3.3 Effect of Cu and Me-JA treatment on non-enzymatic antioxidants

Vitamin are accountable for nutritional value of crop along with building up tolerance power of plant towards any stress. In present research, vitamins *viz.* vitamin A, C and E were evaluated in seedlings of *C. cajan* raised from Me-JA primed seeds under Cu. Data regarding Vitamin A (µg g^{-1}FW) is presented as bar graphs in **Table 1 and Fig 5.**

Table 2: effect of Me-JA and Cu treatment on non enzymatic antioxidants in 15 days old *Cajanus cajan* seedlings

Treatments	Vitamin A (µg gram^{-1}FW)	Vitamin C (µg gram^{-1}FW)	Vitamin E (µg gram^{-1}FW)	Antioxidant Potential (% inhibiton)
Control	35.51±2.50^{a}	448±5.84^{d}	38.4±2.36^{k}	17.0±1.0^{b}
5mM Cu	32.3±1.23a	346±5.39^{e}	94.4±4.21^{h}	40.0±1.0.8^{a}
1µM+5mM Cu	39.4±1.42^{a}	1179±3.87^{a}	182.4±4.8^{d}	25.5±0.5^{b}
1nM+5mM Cu	39.9±1.6^{a}	1167±6.15^{b}	144.8±1.4^{f}	30.4±0.85^{b}
1pM+5mM Cu	28.04±1.2^{a}	1106±3.25^{c}	102.4±1.5^{g}	36.0±2.1^{a}

Vitamin A content was decreased to 10% in 5mM while 22% decrease was reported in 1pM Me-JA treated seedlings grown in 5mM Cu stress. Similarly, Vitamin C is one of the major biomolecule which make plant nutritively valuable and also help in stress management of the plant as an antioxidant. (**Fig. 6, Table 2).** Vitamin

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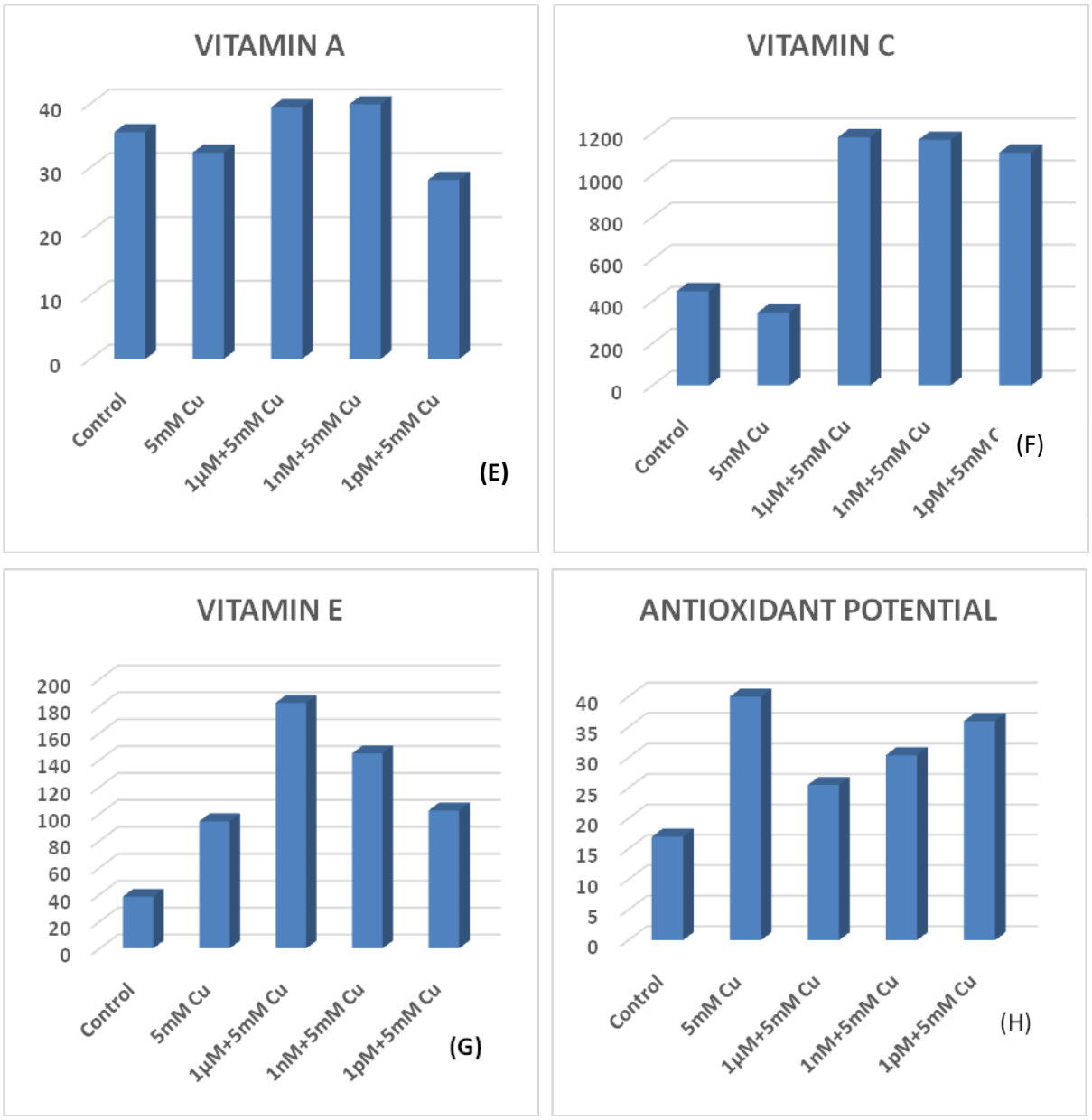

Figure (E-H): effect of Me-JA and Cu treatment on non enzymatic antioxidants of 15 days old *Cajanus cajan* seedlings

C content was also decreased to 23% and increased in Me-JA treated seedlings grown in Cu stress where 1µM Me-JA treated seedlings grown in Cu stress showed 163% enhancement. Wolucka *et al*. [28] documented that induction of AsA in presence of JA may be due to overexpression of two late methyl jasmonate responsive genes involved in AsA biosynthesis.

Vitamin E also called tocopherol (TOCs) are lipophilic antioxidants synthesized by all the plants and are essential components of biological membranes [29]. In higher plants, chloroplast membrane containing TOCs were also known to protect lipids and other membrane components by physically quenching and chemically reacting with O_2 in chloroplasts thus protecting the PSW structure and functions [30]. A very augmenting upshot in vitamin E level was observed in Me-JA primed seedlings (**Fig 7** and **Table 2). Vitamin E** content was increased to 145% as compared to control in 5mM Cu. Upto 375% increase was reported in 1µM Me-JA treated

seedlings grown in Cu stress. DPPH activity was used to check the antioxidant potential of plant raised under different circumstances. In present work, antioxidants potential of 15 days old seedlings was noticed by DPPH assay, in JA or Me-JA treatments with or without Cu concentrations (**Fig. 8** and **Table 2).**

CONCLUSION

This study highlighted the effect of Me-JA seed priming on increasing the oxidative stress tolerance of Pigeonpea seedlings under Cu stress conditions. Pigeonpea seedlings grown in 5mM Cu stress showed highest in crese in lipid peroxidation which was ameliorated with the activation of antioxidant defense system. Antioxidants such as superoxide dismutase, catalase, vitamin E also increase the oxidative stress tolerance of the plant.

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