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Amelorative roles 0f melatonin and sugar beet pulp pellets on pb-stressed moringa oleifera plants

Marwa F. A. El-Sakaan*, Mahmoud E. Younis, Wafaa M. Shukry
Botany Department. Faculty of Science, Mansoura University
*marwafawzi@mans.edu.eg

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Abstract: Pot experiments were conduc.0. 0ed to evaluate the effect of root and foliar uptake of Pb (100 and 400 ppm) on *Moringa oleifera* plants. Furthermore, the possible role of melatonin (MEL) and sugar beet pulp pellets (SBP) in ameliorating lead toxicity was investigated. The growth parameters were declined in case of soil or foliar Pb application. The foliar lead application revealed more negative effect on the growth of moringa plants than the soil application. A decline in proline and oxidative markers was observed in response to Pb stress. Also, Pb stress induced an increase in anthocyanins, ascorbic acid (AsA), total antioxidant capacity (TAC) and a decrease in reduced glutathione (GSH), DPPH-scavenging activity, as compared with the control values. The activities of ascorbate peroxidase (APX), superoxide dismutase (SOD) and glutathione reductase (GR) were negatively correlated with Pb treatments in soil and foliar application. It was found that supplemental addition of MEL or SBP showed an increase in the growth parameters and proline compared to Pb-stressed moringa plants. Moreover, there was variable changes in hydrogen peroxide, lipid peroxidation and electrolyte leakage under MEL and SBP treatments as compared with Pb-stressed plants but still less than the control values. The activities of ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) were improved by MEL and SBP application. The ameliorating effect of SBP against lead toxicity was more pronounced than that of MEL.

Key words: Lead, *Moringa oleifera*, melatonin, sugar beet pulp, antioxidative system

Introduction

Lead is one of the most toxic and frequently encountered [1, 2]. Despite a long history of its beneficial use by humankind, lead has no known biological function in living organisms [3]. Negative impacts on germination and growth occur when plants are exposed to lead, even at micromolar levels [4]. Germination is sharply inhibited by very low concentrations of Pb^{2+} [5, 6]. Lead affects the growth of roots and aerial plant components at low doses [4, 6]. The root has a stronger inhibitory effect, which may be correlated to its higher lead content[7].

Moringa oleifera Lam is one of the most well-known, widely distributed, and naturalized species of a monogeneric family *Moringaceae* [8, 9]. Moringa is a multi-purpose plant that can be utilized as field or fodder crop, crop growth enhancer, biopesticides, biogas, water purification, phytomedical source...etc.[10, 11]. All parts of the *Moringa oleifera* plant have traditionally been utilized for various purposes, but leaves are generally the most commonly used [12, 13]. Moringa leaves have been reported to be a rich source of beta-carotene, protein, vitamin C, calcium and potassium as well as being a strong source of natural antioxidants. In addition, it contains vitamin B complex, chromium, copper, magnesium, manganese, phosphorus and zinc[14]. Several bioactive compounds were detected in the leaves of *Moringa oleifera*. They are categorized as vitamins, carotenoids, polyphenol, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins and oxalates and phytates.

Melatonin (N-acetyl-5-methoxytryptamine) is naturally occurring bioactive indole amine molecule, present in phylogenetically distinct organisms. MEL is involved in a variety of physiological actions in plants, including growth and metabolism regulation [15], improves the growth [16], lateral or adventitious root induction[17, 18], delays leaf senescence [19], heavy metal tolerance[20], alleviates the negative effects of cold stress in melon[21], enhanced drought stress tolerance [22, 23], improving seed germination under salt stress [24] , scavenges the ROS and thus inhibits electrolyte leakage resulting in improving electron transport chain [25].

Sugar beet pulp (SBP) is the fibrous, energy rich by-product resulting from the water extraction of sugar contained in the root of the sugar beet (*Beta vulgaris* L.). In the world, 86% of sugar beet roots are processed into sugar and yield sugar beet pulp [26]. Complete bio-recycling of these agro-industrial wastes is increasingly recognized as a critical component of long-term agriculture sustainability [27]. Furthermore, Caravaca, Alguacil [28] found that adding sugar beet waste to the rhizosphere of *Cistus albidus*L. and *Quercus coccifera*L. enhanced total carbohydrates and soluble C-fraction (water-soluble C and water-soluble carbs).

Plant materials and growth conditions

The experimental work was run with homogeneous lots of moringa (*Moringa oleifera*). Pure strains of seeds were obtained from Ministry of Agriculture, Mansoura, Egypt. Two experiments (A and B) were carried out. The first experiment (A) included the growth of seeds in contaminated soil with lead (100 and 400 ppm $Pb(NO_3)_2$ either alone or in combination with melatonin (100 µM), as a priming solution, or sugar beet pulp pellet, as an amendment, at a ratio of 10g/kg soil according to Ogundiran, Mekwunyei [29]**.** The second experiment (B) included the spraying of 100 days old plant leaves with lead (100 and 400 ppm Pb $(NO_3)_2$ either alone or in combination with melatonin (100 µM) or with sugar beet pulp pellet. Equal amounts (8 kg) of homogeneous mixture of variously treated sand: clay soil (2:1, v/v) were weighed in black polythene bags; these bags being divided into an appropriate replicated number of experimental groups according to the type of the experiment which was conducted as follows: Control (without treatment); 100 ppm Pb $(NO_3)_2$- contaminated soil ;400 ppm Pb $(NO_3)_2$- contaminated soil ; Sugar beet pulp pellet- enriched soil ; 100 ppm Pb $(NO_3)_2$- contaminated soil + sugar beet-enriched soil ; 400 ppm Pb $(NO_3)_2$- contaminated soil + sugar beet-enriched soil. After incubation of the bags with soil for two weeks at normal day and night condition [29, 30], a suitable number of uniform sterilized *Moringa oleifera* seeds were sown in the variously prepared soils.

Data collection

In both experiments, samples were taken for analyses after 110 days from the date of sowing the seeds. Sampling was carried out in a way to include all plants allocated for each treatment. The collected samples were used for assessment of the growth parameters (root and shoot length; fresh and dry weights of root and shoot; water contents). Triplicate samples were taken for, proline, oxidative parameters and antioxidant capacity. The chemical constituents of leaves for moringa were determined. Proline was determined according to the methods of Bates, Waldren [31]. Hydrogen peroxide was estimated according to Alexieva, Sergiev [32]. Lipid peroxidation was estimated by determination of the content of the product of unsaturated fatty acid peroxidation, malondialdehyde (MDA), following the method of Heath and Packer [33]. Electrolyte leakage (E.L) was determined according to Lutts, Kinet [34]. Anthocyanins were determined according to Mirecki and Teramura [35]. Ascorbic acid content was assayed as described by Omaye, Turnbull [36].reduced glutathione was estimated according to [37, 38]. The total antioxidant activity was evaluated as described by Prieto, Pineda [39] and adopted by Pan, Zhu [40]. DPPH-scavenging activity was estimated according to the method of Siger, Czubinski [41]. The activity of catalase was determined by the method of Sinha [42].Ascorbate peroxidase activity was assayed according to Nakano and Asada [43]. Glutathione reductase was assayed according to Goldberg and

Spooner [44]. SOD activity was determined according to the method of Nishikimi, Rao [45].

Statistical analysis

The full data of the variously treated moringa plants were statistically analyzed using one-way analysis of variance (ANOVA) and comparison among means was carried out by calculating the least significant difference test (L.S.D.) at 5% probability level. All the analyses were made using CoStat software (version 6.400).

Results and Discussion

As shown in table 1, a significant reduction in all the measured growth parameters in Pb-treated plants was, in general, observed at the two concentrations of Pb, as compared with the control values. This decrease was more severe in the plants treated with 100 ppm Pb in soil and foliar application. For Pb soil application, the % of decrease in length, fresh weight and dry weight of shoot was 12.30, 21.00 and 26.84, respectively, as compared with controls. These parameters were lowered by 11.32, 28.87 and 36.20% in case of foliar application, as compared with control. The % of change in root length, fresh weight and dry weight in case of soil application was as follows: 11.90, 42.66 and 50.16, respectively. For foliar application, the % of decrease was 15.48, 53.40 and 57.56 in root length, fresh weight and dry weight, respectively. As compared with the control values, the water content markedly decreased in the stressed plants by 29.57 and 39.08% in case of 100 ppm Pb soil and foliar application and by7.90 and 17.75% in case of 400 ppm Pb soil and foliar application, respectively. Similar results have already been reported under root uptake in water hyacinths [46], *moringa oleifera* [47] and after foliar application of Pb in radish [48] and *Spinacia oleracea*[49].

In response to MEL and SBP treatment an increase in all the growth parameters was apparent compared to Pb-alone treatment; the magnitude of increase being more pronounced under beet application. Our findings are in line with those of Xie, Xiong [50] who reported that the pretreatment with melatonin at 100 µM reduced the damages caused by Pb and resulted in longer shoot and root lengths and higher biomass accumulations compared to stressed bermudagrass plants. In accordance with our results, Caravaca, Alguacil [28] observed that the application of sugar beet, rock phosphate, and *Aspergillus niger* directly into soil and the mycorrhizal inoculation of seedlings can considerably enhance the growth of *Cistus albidus* L. and *Quercus coccifera* L.

In accordance with figure 1, proline content significantly decreased in all plants exposed to Pb stress by 50.52 and 40.03% in case of 100 and 400 ppm Pb soil application and by 31.92 and 29.88 % in case of 100 and 400 ppm Pb foliar treatment, respectively, as compared with the control values. Several studies have shown a decrease in proline in *Raphanus sativus* stressed by zinc at dosages of 0 and 100 ppm [51], as well as in *Brassica juncia* exposed to high levels of lead and cadmium [52].

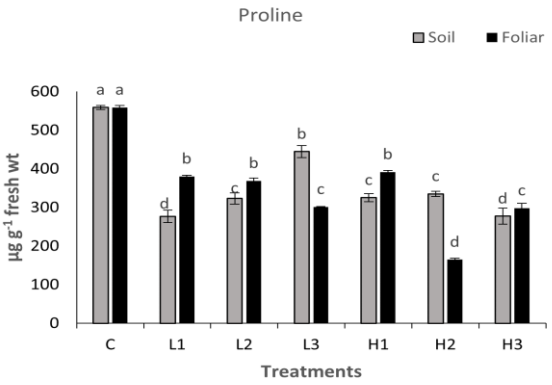

Fig 1: Effect of lead stress either alone or in combination with MEL or SBP on proline of *Moringa oleifera* plants. C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Vertical bars represent standard error.

Application of MEL or SBP elicited an increase in proline content at 100 ppm Pb and a decrease at 400 ppm Pb in case of soil application compared to Pb-stressed plants. on the other hand, it was observed that application of MEL or SBP showed, in general, a decline in proline content in case of soil or foliar treatment except for the moringa plants treated with 100 ppm Pb in soil showed a significant increase in proline content. Similarly, Xie, Xiong [50] observed that pre-treatment with100 µM melatonin inhibited the accumulation of proline under Pb stress. In contrast, Zhang,

Zhang [53] reported that MEL application promoted the accumulation of proline under Pb stress. On the other hand, it was found that mycorrhizal plants grown in the SBP-amended soil reached the highest proline were the least-damaged (in terms of plant growth) by drought [54].

As illustrated in figure 2, the content of hydrogen peroxide significantly decreased by 7.08 and 41.67% at 100 and 400 ppm Pb soil application, respectively, as compared with control. The foliar application of 100 ppm Pb caused significant reduction in H_2O_2 content by 45.52% below the control value. However, it was observed that 400 ppm Pb treatment caused a significant increase in the content of hydrogen peroxide by 15.38%, as compared with control. It is clear in figure 2 that MDA content significantly decreased under Pb stress in both cases of soil and foliar application, as compared with the control values. The % of decrease at 100 and 400 ppm Pb was 26.17 and 42.49%, respectively, for soil treatment as well as 70.84 and 53.00% for foliar treatment. Also, Pb stress caused a significant decrease in E.L by 6.00 and 8.36% for soil application and by 9.88 and 3.27% for

foliar application at 100 and 400 ppm Pb treatment, respectively. In accordance with our results, Sofy, Seleiman [55] reported the exposure of Para Grass to high concentration of Pb decreased H_2O_2 content and MDA level, as compared with control. MDA and H_2O_2 levels in *S. grandiflora* have similarly to decrease with increasing Pb concentrations to a certain level [56]. MEL-pretreated plants showed, in general, an increase in hydrogen peroxide content, MDA and E.L in case of Pb foliar application and in the 100 ppm Pb-stressed plants applied in soil.

Table 1: Effect of lead stress either alone or in combination with MEL or SBP on growth parameters (shoot and root length; cm plant^{-1}, shoot and root fresh weight; g plant^{-1}, shoot and root dry weight; g plant^{-1}, water content; g plant^{-1}) of Moringa oleifera plants.

	Treatment	Shoot			Root			W.C
		length	F. wt.	Dry wt.	length	F. wt.	Dry wt.	
Soil application	Control	94.83 ^{c} ± 1.48	30.28 ^{d} ± 1.59	6.67 ^{b} ± 0.34	14.00 ^{ab} ± 0.58	28.57 ^{a} ± 1.34	6.44 ^{a} ± 0.30	45.74 ^{c} ± 2.30
	100 ppm Pb $(NO_3)_2$	83.17 ^{e} ± 1.01	23.92 ^{e} ± 1.74	4.88 ^{c} ± 0.28	12.33 ^{c} ± 0.67	16.38 ^{d} ± 0.36	3.21 ^{d} ± 0.01	32.22 ^{d} ± 1.80
	+MEL	83.50 ^{e} ± 2.93	20.44 ^{e} ± 1.20	5.92 ^{bc} ± 0.98	12.57 ^{bc} ± 0.52	23.60 ^{b} ± 0.61	5.30 ^{b} ± 0.15	32.82 ^{d} ± 1.06
	+Beet	115.17 ^{a} ± 0.88	48.54 ^{b} ± 1.57	9.82 ^{a} ± 0.59	13.83 ^{abc} ± 0.73	28.85 ^{a} ± 0.35	5.83 ^{b} ± 0.26	61.74 ^{a} ± 1.16
	400 ppm Pb $(NO_3)_2$	89.37 ^{d} ± 0.73	35.01 ^{c} ± 0.98	7.40 ^{b} ± 0.16	12.57 ^{bc} ± 0.28	18.76 ^{c} ± 0.38	4.25 ^{c} ± 0.06	42.13 ^{c} ± 0.49
	+MEL	102.00 ^{b} ± 1.15	47.46 ^{b} ± 0.60	10.06 ^{a} ± 0.24	12.93 ^{abc} ± 0.29	18.32 ^{cd} ± 0.28	3.67 ^{d} ± 0.10	52.05 ^{b} ± 0.47
	+Beet	119.00 ^{a} ± 0.58	55.47 ^{a} ± 2.49	10.68 ^{a} ± 0.57	14.50 ^{a} ± 0.50	18.89 ^{c} ± 0.54	3.74 ^{cd} ± 0.21	59.94 ^{a} ± 2.24
	l.S.D	4.408	4.721	1.581	1.628	1.968	0.562	4.659
Foliar application	Control	94.83 b ± 1.48	30.28 c ± 1.59	6.67 b ± 0.34	14.00 b ± 0.58	28.57 a ± 1.34	6.44 a ± 0.30	45.74 b ± 2.30
	100 ppm Pb $(NO_3)_2$	84.10 e ± 1.25	21.54 e ± 0.77	4.26 d ± 0.03	11.83 c ± 0.60	13.31 d ± 0.38	2.73 d ± 0.15	27.86 d ± 1.01
	+MEL	89.33 cd ± 1.01	26.39 d ± 0.28	5.14 c ± 0.25	14.67 b ± 0.88	18.86 c ± 1.11	3.77 c ± 0.59	36.34 c ± 0.55
	+Beet	102.77 a ± 2.15	36.72 b ± 0.35	7.09 b ± 0.23	19.00 a ± 1.00	19.55 c ± 1.06	3.81 c ± 0.34	45.37 b ± 0.69
	400 ppm Pb $(NO_3)_2$	84.99 de ± 1.30	26.19 d ± 1.08	5.82 c ± 0.20	12.83 bc ± 0.60	22.25 b ± 0.43	4.99 b ± 0.06	37.62 cd ± 0.96
	+MEL	90.37 bc ± 0.82	27.16 d ± 0.09	5.41 c ± 0.27	14.67 b ± 0.67	20.78 bc ± 0.50	4.54 bc ± 0.11	37.98 c ± 0.79
	+Beet	106.67 a ± 2.03	43.28 a ± 0.61	8.73 a ± 0.14	18.23 a ± 0.39	20.16 bc ± 0.27	4.46 bc ± 0.12	50.25 a ± 0.35
	l.S.D	4.567	2.526	0.695	2.124	2.514	0.890	3.392

Values listed represent the means ± standard error. Different superscript letters refer to significant variation; with the least significant difference (LSD) at $p \leq 0.05$.

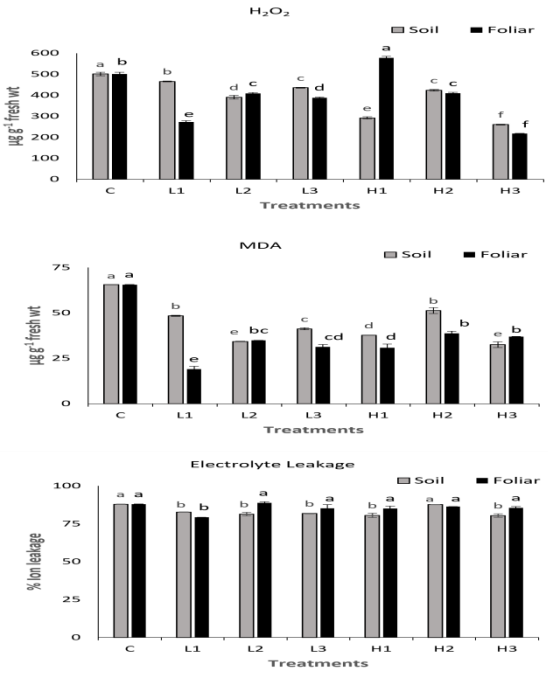

Fig 2: Effect of lead stress either alone or in combination with MEL or SBP on hydrogen peroxide, lipid peroxidation and electrolyte leakage of *Moringa oleifera* plants. C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Vertical bars represent standard error.

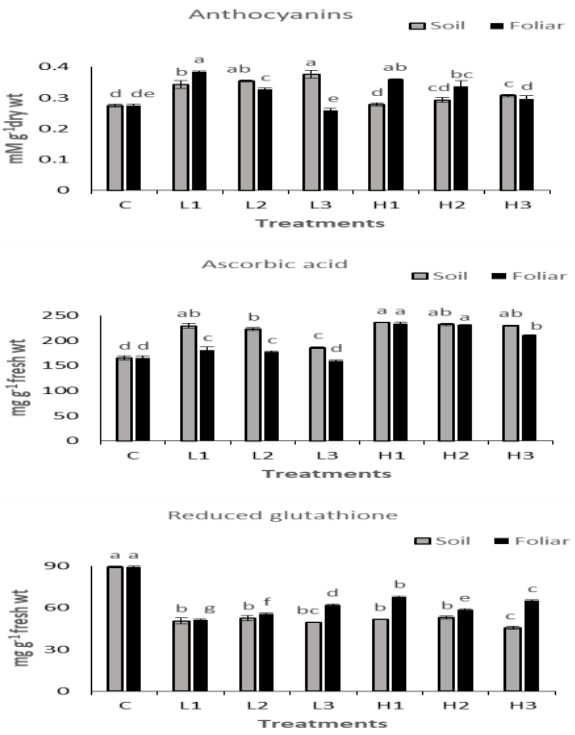

Fig 3: Effect of lead stress either alone or in combination with MEL or SBP on antioxidant compounds of *Moringa oleifera* plants. C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Vertical bars represent standard error.

Supplemental addition of SBP to Pb-stressed plants showed, in general, a decline in H_2O_2, MDA and E.L compared to stressed moringa plants in case of soil application and an increase in these oxidative markers in case of foliar application. Many researchers revealed considerable reductions in reactive oxygen species accumulation, MDA content and E.L level in watermelon, tomato, soybean, maize and by MEL supplementation, under abiotic stresses [57-60]. Anli, Baslam [61] observed that the application of single or combined biofertilizers under water stress elicited reduced MDA and H_2O_2 content compared to non-amended controls.

Figure 3 shows that anthocyanins and AsA content significantly increased by 25.18 and 38.72% at 100 ppm Pb stress and by 1.69 and 42.54% at 400 ppm Pb in case of soil application, respectively. In case of foliar application, the % of increase in anthocyanins and AsA content was 40.19 and 9.56% at 100 ppm Pb as well as 31.07 and 41.64% at 400 ppm Pb. In contrast, it was recorded significant decreases in GSH content at 100 ppm Pb by 43.38and 42.16 at 400 ppm Pb in case of soil application compared to the control values. In case of foliar application, the % of decrease in GSH content was 42.54 at 100 ppm Pb and 24.07 at 400 ppm Pb. MEL pre-treatment showed non-significant changes in anthocyanins, AsA and GSH contents compared to Pb-stressed moringa plants in case of soil application. In foliar application, MEL application caused, in general, decreases in anthocyanins, AsA and GSH contents. On the other hand, SBP application caused a significant decrease in AsA and GSH content when Pb applied in soil or by foliar treatment and in anthocyanins content in case of foliar application only. In the work of Wu, Wang [62], Cd stress increased the anthocyanin content of strawberry seedlings and that melatonin stimulated anthocyanin accumulation. The activity of two important antioxidants, AsA and GSH, altered greatly under Pb stress. In the study of Hasanuzzaman, Matin [63], lead stress reduced the AsA content in a dose-dependent manner, whereas the GSH

content increased. Another study showed an increase of AsA in groundnut cultivars growing in the

presence of Pb-stress[64]. Lead-induced alterations in GSH have also been shown in *Acacia farnesiana* [65] and *Talinum triangulare* [66]. On the other hand. it was observed that pre-treatment with melatonin significantly increased the levels of AsA and GSH according to Xie, Xiong [50]. These findings are in line with those who reported the effects of melatonin on drought stressed *Triticum aestivum* L. [67], salt stressed *Citrullus lanatus* L. [68], and Cd-stressed*Malachium aquaticum* and *Galinsoga parviflora* [69].

As shown in figure 4, in response to treatment with 100 and 400 ppm Pb, the total antioxidant capacity appeared to increase significantly by 16.45 and 11.89% for soil application and by 2.29 and 10.48% for foliar application, respectively, as compared with control. As shown in figure 4, the plants exposed to Pb stress possessed a significant decrease in DPPH-scavenging activity below those of controls in case of soil and foliar application. The % of decrease was 7.55 and 8.66% at 100 and 400 ppm Pb for soil application as well as 6.21 and 3.52 % for foliar application, respectively. In general, MEL pre-treatment showed an increase in TAC and DPPH-scavenging activity under 100 and 400 ppm Pb stress in case of soil and foliar application. Moreover, supplemental addition of SBP induced a non-significant increase in TAC and significant increase in DPPH-scavenging activity in case of and foliar soil application.

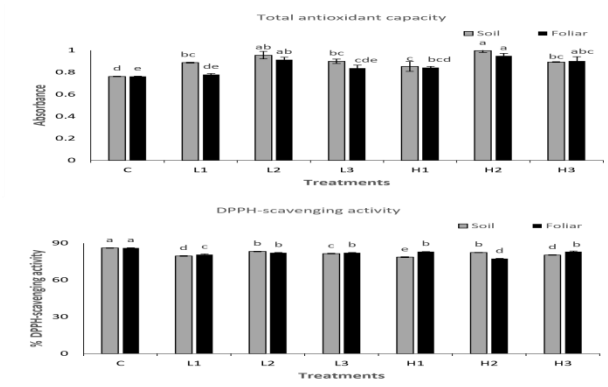

Fig 4: Effect of lead stress either alone or in combination with MEL or SBP on antioxidant capacity of *Moringa oleifera* plants. C; control, L1; 100 ppm Pb, L2; 100 ppm Pb + MEL, L3; 100 ppm Pb + SBP, H1; 400 ppm Pb, H2; 400 ppm Pb + MEL, H3; 400 ppm Pb + SBP. Vertical bars represent standard error.

In accordance with our results, Azeez, Adejumo [47] reported that Cd and Pb-induced toxicities led to significant reduction in DPPH scavenging activity and compared to the control. Also, a decline in DPPH-scavenging activity was recorded for maize plants under cadmium stress compared to control plants[70]. In accordance with our results, it was recorded an increase in total antioxidant capacity in *Plantago ovata* under Cr (VI) stress[71], and in *Brassica juncea* under cadmium stress[72].

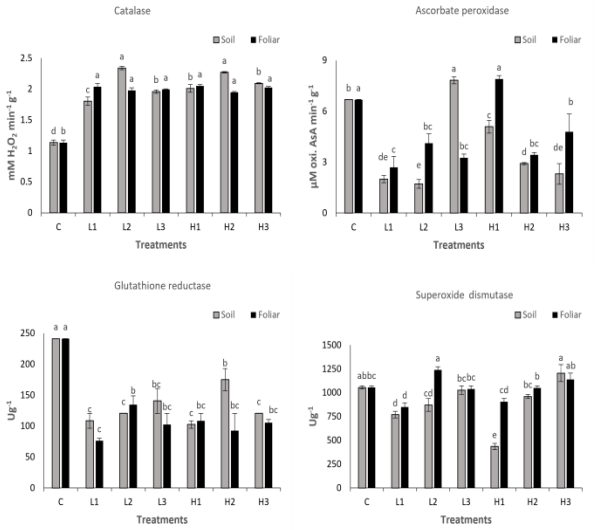

Fig 5 shows the activity of CAT, APX, GR and SOD enzymes of the control and the variously treated moringa plants. As compared with the control values, the stress, as generated by Pb, significantly increased the activity of CAT enzyme by 58.98 and 77.16% under 100 and 400 ppm Pb soil treatment as well as by 79.84 and 80.86% in case of foliar treatment, respectively. On the other hand, significant decreases in APX, GR and SOD activities were observed below the control values in case of soil and foliar application. The % of decrease was 70.11, 55.00 and 26.98% at 100 ppm Pb for soil application and 23.89, 57.50 and 58.73% under 400 ppm Pb treatment. The % of change was 59.78, 68.33 and 19.58% at 100 ppm Pb for foliar application compared to the control and 18.19, 55.00 and 12.90% at 400 ppm Pb in APX, GR and SOD activities, respectively. The combination of MEL or SBP

with Pb stress caused a significant increase in CAT activity in case of soil application. However, supplemental addition of MEL or SBP to Pb-stressed plants showed a non-significant change in CAT activity in case of foliar application. In general, marked increases in GR and SOD activities were recorded when MEL or SBP combined with Pb stress in both cases of soil and foliar application. On the other hand, MEL and SBP application showed an increase in APX activity in case of 100 ppm Pb treatment for soil and foliar treatment and a significant decline under 400 ppm Pb stress. In study of **[73],** SOD activity decreased among treatments compared to control, while CAT activity increased steadily with increasing cadmium concentration. Other studies reported an increase in the activity of CAT, SOD and APX under Pb stress in *Ceratophyllumdemersum* [74], APX, CAT and GR in *Triticum aestivum* [75]. Our findings are in line with those Wang, Duan [76] who reported that pre-treatment with melatonin significantly increased the activities of SOD, CAT, APX, and GR.

Conclusion.

The present study showed that Pb stress resulted in reduced biomass production. The adverse effect of the foliar lead application on moringa plants was higher than the soil treatment. On the other hand, application of MEL and SBP consistently increased the chlorophyll content and improved the antioxidative system and subsequently biomass accumulations. However, supplemental addition of SBP was seen to be more efficient in mitigation of overall stress responses than MEL.

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