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Growth and biochemical responses of moringa (*Moringa oleifera* L.) to vermicompost and phosphate rock under water stress conditions

Crecimiento y respuestas bioquímicas de moringa (*Moringa oleifera* L.) a vermicomposta y roca fosfórica en condiciones de estrés hídrico

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Abstract. The aim of this investigation was to analyze the effect of vermicompost and phosphate rock on plant growth and biochemical responses of *Moringa oleifera* exposed to water deficit and waterlogging conditions. Treatments included 152 g of vermicompost (V) and 1.3 g of phosphate rock (PR) added to each cultivation bag. Control was prepared with 2.3 g of urea. All treatments were irrigated under different watering conditions [water deficit: irrigated at 20% water holding capacity (WHC); control (40% WHC) and waterlogging (60% WHC)]. The overall average in plant height was 90, 86 and 68 cm, whereas chlorophyll was 45, 40 and 39 Spad units under water deficit, control and waterlogging conditions, respectively. Proline concentration in *M. oleifera* leaves was higher in the V+PR treatment under water deficit conditions. Catalase and peroxidase activity were higher in plants cultivated under waterlogging conditions. Enzyme activities on plants cultivated under water deficit were not different in comparison to control plants. In conclusion, vermicompost was able to minimize the harmful effects of waterlogging. The application of vermicompost and phosphate rock in *M. oleorifera* favored the synthesis of catalase and peroxidase, and inhibited the accumulation of reactive oxygen species (ROS), thus protecting the plant from membrane damage and oxidative stress under waterlogging conditions.

Keywords: Catalase; Chlorophyll; Vermicompost; Proline; Peroxidase.

Resumen. El objetivo de esta investigación fue analizar el efecto de la vermicomposta y la roca fosfórica sobre el crecimiento de la planta y respuestas bioquímicas de moringa (*Moringa oleifera* L.) cultivada en condiciones de déficit hídrico y anegamiento. Los tratamientos fueron: 152 g de vermicomposta (V) y 1,3 g de roca fosfórica (RF) adicionados por cada bolsa de cultivo. El control se preparó con 2,3 g de urea. Todos los tratamientos fueron irrigados con diferentes condiciones [déficit hídrico: riego con 20% de capacidad de retención de agua, CRA), control (40% CRA) y anegamiento (60% CRA)]. El promedio general en altura de la planta fue 90, 86 y 68 cm, mientras que la clorofila tuvo 45, 40 y 39 unidades de Spad en condiciones de déficit hídrico, control y anegamiento, respectivamente. La concentración de prolina en las hojas fue mayor en el tratamiento V+PR en condiciones de déficit de agua. La actividad de catalasa y peroxidasa fue mayor en las plantas cultivadas en condiciones de anegamiento. Las actividades enzimáticas en plantas cultivadas con déficit hídrico no fueron diferentes en comparación con las plantas control. En conclusión, la vermicomposta fue capaz de minimizar los efectos nocivos del exceso de agua en el cultivo de moringa. La aplicación de vermicomposta y roca fosfórica, favoreció la síntesis de catalasa y peroxidasa, inhibió la acumulación de especies reactivas de oxígeno (ROS), y protegió a la planta del daño a la membrana y el estrés oxidativo en condiciones de anegamiento.

Palabras clave: Catalasa; Clorofila; Vermicomposta; Prolina; Peroxidasa.

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INTRODUCTION

Moringa oleifera Lam is a plant with medicinal uses and high nutritional value. Different parts of this plant contain important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and phenolics compounds (Anwar et al., 2007). Also is an acceptable feedstock for biodiesel (Rashid et al., 2008). Another interesting application is for use as biosorbent in water decontamination, and treatment of industrial and agricultural wastewaters (Akhtar et al., 2007). *Moringa* growers have found that plants become stressed and even die when plants have excess water. It was reported a study to assess whether recurring water stress occurring from seed germination to young plants of *M. oleifera* were able to mitigate the drought stress effects. The results suggested that seeds of *M. oleifera* subjected to mild water deficit increased the ability for drought tolerance on young plants of this species (Rivas et al., 2013). Glycine betaine (GB) and proline are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals. Both compounds are thought to have positive effects on enzyme and membrane integrity (Ashraf & Foolad, 2007). Based on peach fruit (*Prunus persica* L.) results, it appears that a higher unsaturation degree of membrane lipid and NAPE (N-acylphosphatidylethanolamine) accumulation are beneficial for maintaining membrane fluidity, leading to an enhanced tolerance of peach fruit to chilling stress (Zhang & Tian, 2010).

Currently, conventional agriculture has been characterized by an excessive use of chemical fertilizers, herbicides and pesticides (Roos et al., 2018), which leads to serious environmental problems, such as salinization, degradation of soil and loss of water retention capacity (Aguilar-Benitez et al., 2017). To address these problems, an alternative is the use of vermicompost as an organic fertilizer. Different studies (Kaur et al., 2018) have been established to investigate the amendment effects of vermicompost. Thus, vermicompost (V) is thought to improve soil quality and plant yield, and be an effective fertilizer with high water-holding capacity. Therefore, the potential is considered very high for V and crop cultivation. In addition, Zuo et al. (2018) reported that the application of vermicompost improves strawberry growth and quality through increased photosynthesis rate, free radical scavenging and soil enzymatic activity. Hosseinzadeh et al. (2016) evaluated the effects of V on photosynthetic activity of chickpea (*Cicer arietinum* L. cv. Karaj) under drought conditions and found a positive effect of the V on photosynthesis under no stress conditions. However, these results were not found under moderate and severe drought conditions. In barley production under water deficit conditions, grain mineral quality could be improved through vermicompost and NPK integrated fertilizer application (Farahani et al., 2011). It is not yet known whether water deficit or waterlogging could be induce changes in *Moringa* plant growth, chlorophyll, proline concentration and enzyme activity, and if vermicompost or phosphate rock could help to reduce the stress caused by water deficit or waterlogging conditions. The aim of this work was to analyze the effect of vermicompost and phosphate rock on plant growth of *Moringa oleifera* Lam, cultivated under water deficit and waterlogging conditions, and to evaluate the changes in chlorophyll, proline, and stress enzymes.

MATERIALS AND METHODS

Plant material. *Moringa oleifera* seeds were soaked in bags with a diameter of 30 cm and 60 cm length containing 9 kg of soil each. In treatment with vermicompost, 152 g of vermicompost (V) was added in each bag. In phosphate rock treatment, 1.3 g of phosphate rock (PR) was added, whereas that in V+PR treatment, 152 g of vermicompost and 1.3 g of phosphate rock were added. A control treatment was prepared with 2.3 g of urea. All treatments were irrigated under different watering conditions (water deficit: irrigated with 0.75 L, 20% water holding capacity); control (irrigated with 1.5 L, 40% water holding capacity); waterlogging (irrigated with 2.2 L, 60% water holding capacity). Seed germination occurred between 10 to 20 days after soaking. The seeds do not require pre-germination treatments because of their high germination rates, greater than 90%.

Vermicompost and phosphate rock. Cow manure vermicompost was obtained from the Luanda organic farm located in Ocozocoautla-Villaflores (Chiapas, México). Further details of the vermicomposting process can be found in Gutiérrez-Miceli et al. (2008). Rock phosphate was obtained from 'Calizas Industrializadas de Hidalgo' (Pachuca, Hidalgo, México).

Soil and vermicompost characterization. Details of the techniques used to measure electrolytic conductivity (EC), pH, total C, Available phosphorus, total N, water holding capacity (WHC), humidity and textural characteristics of the soil and vermicompost can be found in Gutiérrez-Miceli et al. (2008) and Ruiz-Valdiviezo et al. (2010).

Plant height. Plant physiological measurements included plant height (cm) and stem diameter (mm), which were measured using a standard meter stick.

Chlorophyll. Leaf chlorophyll content was measured fifteen days after plant germination using a SPAD-502 meter (Minolta Corporation, Ramsey, NJ) and expressed as SPAD units. Each reported value was the average of six readings per plant, i.e. three measurements in each side of leaf midrib, and using leaves from the top, bottom, and middle on each plant.

Determination of free proline. For determination of proline, 400 mg of moringa leaves were homogenized and centrifuged (13000 rpm) during 10 min with 3 mL of aqueous solution of sulfosalicylic acid at 3% (Bates et al., 1973). A small volume (200 µL) of the supernatant was thereafter transferred to a test tube where we added 400 µL of ninhydric acid and 2 mL of glacial acetic acid. This was incubated at 100 °C for one hour. Thereafter, the reaction was stopped in an ice bath. Then, 2 mL of toluene were added and the content of the tube was inverted during 30 seconds. After this, the toluene phase was sucked using a pipette and kept at room temperature to stabilize. Finally, its absorbance was determined at 520 nm with a visible light spectrophotometer Thermo Scientific Genesys 20, Germany. The results were interpolated using a proline standard curve to determine the amount of proline in leaves (µg/g of leaf dry weight).

Analysis for enzymes

Preparation of enzyme extract from leaves of Moringa oleifera. After 3 days of treatment, the leaf tissues (500 mg) were ground in a cold mortar with 3 mL phosphate-Na buffer (100 mM, pH7.0) containing 5 mM EDTA following Dawei et al. (2012). The homogenate was centrifuged at 6000 rpm for 15 min at 4 °C, and the collected supernatant was used for the assays of catalase (CAT) and peroxidase (PX), respectively.

Analysis of catalase activity. The catalase activity was determined spectrophotometrically, based on the method proposed by Aebi (1984), by measuring the rate of decrease in H_2O_2 absorbance at 240 nm at room temperature. One hundred microliter of the supernatant previously obtained was mixed with 1900 µL of 10 mM of Tris-HCl buffer pH 8.0 and 200 µL of 100 mM H_2O_2. The change in absorbance of the each sample at 240 nm was monitored for 60 s. The enzyme activity was defined as units of enzyme activity per mg of protein (U/mg P), where each U is equal to the decomposition of 1 µmol/min of H_2O_2 (Chen et al., 2010).

Analysis of peroxidase activity. The activity of peroxidase was evaluated by adding 50 µL of the supernatant (crude enzyme preparation) to 2 mL of a solution containing 50 mM potassium phosphate buffer pH 6.8, 20 mM guaiacol and 20 mM H_2O_2. After incubation at 30 °C for 10 min, the reaction was stopped by adding 0.5 mL 5% (v/v) H_2SO_4, and the absorbance was read at 480 nm (Cavalcanti et al., 2004). One POX unit was defined as the change of 1.0 absorbance unit per ml enzymatic extract, and expressed as units of enzyme activity per g dry matter per min (UA/g DW/min).

Statistical analysis. Significant differences for plant height, chlorophyll contents in leaves, proline and catalase because of the different treatments were determined by analysis of variance (ANOVA). Mean comparisons were made using the least significant difference test. The general linear model procedure was used (PROC GLM SAS Institute, 1989).

RESULTS

Physicochemical characteristics of the soil and vermicompost. The values of physico-chemical characterization of soil and vermicompost are given in Table 1. The pH of the agriculture soil was 6.8. This result indicated that a neutral soil was used in this study such as it was reported by Campillo & Sadzawka (2006). This parameter is similar to pH's reported in different studies of agricultural soils in different municipalities in the state of Chiapas (Ruiz-Valdiviezo et al., 2010). The electrolytic conductivity was 0.43 dS/m. It is known (NOM-021-RECNAT-2000) that soils with values of electrolytic conductivities up to 4 dS/m are considered agricultural soils. Additionally, this value indicates the low salinity of this soil, so the fertility was good enough to establish different types of crops.

Table 1. Soil and vermicompost physicochemical characteristics.
Tabla 1. Características fisicoquímicas del suelo y la vermicomposta.

	EC[a]	pH	Total C	Total N	P[b]	WHC[c]
	dS/m		——g/kg——		mg/kg	— % —
Soil	4.3	6.8	9.65	1.8	4.0	41
Vermi compost	8	8.6	233	11.8	1.9	72

[a] EC: Electrolytic conductivity.
[b] P: Available phosphorus .
[c] WHC: Water holding capacity.

Other important parameters were the carbon (19.4 g C/kg ss) and nitrogen (1.9 g N/kg ss) contents. These values were found in the range reported by Ruiz-Valdiviezo et al. (2010). Additionally, this result indicates that the physicochemical characteristics of the agricultural soil such as the C to N ratio was 10.2. This value is ideal for the growth of different plants.

Morphological and physiological traits. Plant height was affected for both the watering conditions and the soil amended treatment (Table 2). Under water deficit conditions, plants grew higher when they were cultivated in soil amended with vermicompost (V), and vermicompost plus phosphate rock (V+PR) treatments. Plant height was 35 cm more in comparison with control plants (soil amended with urea). This trend was similar in control plants (irrigated with 40% soil water holding capacity) and in plants exposed to waterlogging conditions. However, the plant height difference was more evident in control plants (45 cm) than in those exposed to waterlogging conditions (15 cm). The overall average in plant height was 90, 86 and 68 cm in the water deficit, control and waterlogging treatment, respectively. This result indicates that *Moringa oleifera* plants showed more stress under waterlogging than water deficit conditions. However, the vermicompost was able to minimize the harmful effects of waterlogging. Also, the overall average among treatments indicates that the vermicompost was the most effective amendment for promoting an increase (32 cm in comparison with plants fertilized with urea) in plant height.

Also, chlorophyll contents in leaves were affected for both watering conditions and soil amended treatments (Table 3). With water deficit conditions, chlorophyll contents were higher in PR and urea treatments in comparison to V and V+PR treatments. In control plants, chlorophyll contents were higher in PR, V+PR and urea treatments and lowest in the V treatment. In the waterlogging treatment, urea promoted more chlorophyll contents compared to the other treatments (Table 3). Urea and PR additions did not promote changes in chlorophyll contents under the different watering conditions. However, vermicompost and V+PR treatments induced more chlorophyll contents in leaves of *M. oleifera* plants cultivated under water deficit. The overall average in chlorophyll contents were 45.2, 39.1 and 38.9 Spad units under water deficit,

Table 2. Plant height of *Moringa oleifera* L cultivated with Vermicompost (V), Phosphate rock (PR), V + PR and Urea under different water stress conditions.
Tabla 2. Altura de las plantas de *Moringa oleifera* L cultivadas con Vermicomposta (V), Roca fosfórica (RF), V+RF y Urea en diferentes condiciones de estrés hídrico.

	Plant height — cm				
Treatment	**Water deficit**	**Control [1]**	**Waterlogging**	**Overall average**	**LSD[4]**
Vermicompost (V)	[2,3] 105 ± 18.03 a A	101.3 ± 12.86 a A	74.2 ± 1.61 a C	93	25.609
Phosphate rock (PR)	80 ± 2.65 b A	76.7 ± 17.01 b cA	66.5 ± 0.5 bc A	75	19.864
V+PR	105 ± 7.81 a A	105.5 ± 16.12 a A	73.2 ± 3.75 a B	95	21.107
Urea	69.7 ± 15.95 bc A	60.7 ± 8.33 d A	59.3 ± 6.66 d A	63	22.127
Overall average	90	86	68		
LSD (0.05) 4	18.792	18.120	17.011		

[1] Plants irrigated with 40 % soil water retention capacity.
[2] Lowercase letters represent the differences between means by columns.
[3] Uppercase letters represent the differences between means by row.
[4] Least Significative Difference (0.05).

Table 3. Chlorophyll contents in leaves of *Moringa oleifera* L cultivated with Vermicompost (V), Phosphate rock (PR), V + PR and Urea under different water stress conditions.
Tabla 3. Contenido de clorofila en hojas de *Moringa oleifera* L cultivadas con Vermicomposta (V), Roca fosfórica (RF), V+RF y Urea en diferentes condiciones de estrés hídrico.

	Chlorophyll in leaves — SPAD units				
Treatment	**Water deficit**	**Control**	**Waterlogging**	**Overall average**	**LSD [3]**
Vermicompost (V)	42.1 ± 7.0 b[1] A[2]	35.9 ± 4.8 bB	36.2 ± 2.4 bB	38.1	4.96
Phosphate rock (PR)	47.4 ± 3.3 a A	40.5 ± 2.9 a A	38.7 ± 4.3 b A	42.2	3.43
V+PR	42.3 ± 4.9 b A	39.5 ± 6.8 ab B	36.1 ± 2.9 b B	39.3	4.84
Urea	48.9 ± 4.3 a A	40.6 ± 2.7 a A	44.9 ± 5.9 a A	44.8	4.56
Overall average	45.2	39.1	38.9		
LSD [3] (0.05)	4.86	4.43	3.96		

[1] Lowercase letters represent the differences between means by columns.
[2] Uppercase letters represent the differences between means by row.
[3] Least Significative Difference (0.05).

control and waterlogging conditions, respectively. This result indicates that *Moringa oleifera* plants were under more stressful conditions under waterlogging than under water deficit conditions. Only urea was able to minimize the harmful effects of waterlogging. In addition, the overall average among treatments indicates that urea and PR treatments were the amendments more effective to promote an increase in average chlorophyll contents (38.1, 42.2, 39.3, 44.8 Spad units for V, PR, V+PR and Urea, respectively).

Proline concentration in *M. oleifera* leaves was higher in PR and V+PR treatments under water deficit conditions. However, in waterlogging and control plants, proline concentration was not different between treatments (Table 4). Phosphate rock was involved in the changes in proline under the different watering conditions, with respect to the control (urea treatment). The overall average was 0.14, 0.14 and 0.21 under water deficit, control and waterlogging watering conditions, respectively. For vermicompost, phosphate rock, V+PR and urea treatments the overall averages were 0.15, 0.16, 0.19 and 0.15, respectively.

Catalase and peroxidase activity were higher in plants cultivated under waterlogging conditions. Enzyme activities on plants cultivated under water deficit were not different to those found in the control plants (Table 5).

Table 4. Proline concentrations in leaves of *Moringa oleifera* L plants cultivated with Vermicompost (V), Phosphate rock (PR), V + PR and Urea under different water stress conditions.
Tabla 4. Concentraciones de prolina en hojas de *Moringa oleifera* L cultivada con Vermicomposta (V), Roca fosfórica (RF), V+RF y Urea en diferentes condiciones de estrés hídrico.

	Proline in leaves μg/g				
Treatment	Water deficit	Control	Waterlogging	Overall average	LSD [3]
Vermicompost (V)	0.12 ± 0.03 b[1] B[2]	0.13 ± 0.01 a B	0.20 ± 0.05 a A	0.15	0.068
Phosphate rock (PR)	0.13 ± 0.03 ab B	0.15 ± 0.01 a AB	0.19 ± 0.03 a A	0.16	0.05
V+PR	0.18 ± 0.01 a A	0.15 ± 0.07 a A	0.23 ± 0.08 a A	0.19	0.12
Urea	0.11 ± 0.04 b A	0.13 ± 0.05 a A	0.21 ± 0.06 a A	0.15	0.10
Overall average	0.135	0.14	0.11		
LSD[3] (0.05)	0.055	0.08	0.049		

[1] Lowercase letters represent the differences between means by columns.
[2] Uppercase letters represent the differences between means by row.
[3] Least Significative Difference (0.05).

Table 5. Enzymatic activity of catalase (CAT) and peroxidase (POX) in leaves of *Moringa oleifera* L cultivated under different water stress conditions.
Tabla 5. Actividades enzimáticas de catalasa (CAT) y peroxidasa (POX) en hojas de *Moringa oleifera* L cultivada en diferentes condiciones de estrés hídrico.

Treatments	Activity of CAT (U/mg P)	Activity of POX (U/mg P)
Water deficit [1]	0.140 a	6.90 a
Control [2]	0.120 a	7.05 a
Waterlogging [3]	0.185 b	10.7 b
LSD [4] (0.05)	0.028	1.99

[1] Plants irrigated with 20% soil water retention capacity.
[2] Plants irrigated with 40% soil water retention capacity.
[3] Plants irrigated with 60% soil water retention capacity.
[4] Least Significative Difference (0.05).

DISCUSSION

Moringa oleifera plants suffered more stress under waterlogging conditions than under water deficit. Vermicompost was able to minimize the harmful effects of waterlogging and was the amendment more effective for promoting an increase (32 cm in comparison with plants fertilized with urea) in plant height. The results could be explain for several reasons: (1) waterlogging induces hypoxic status in plant cells and the sum result of this metabolic scenario is the accumulation of alanine and succinate and the production of extra ATP under hypoxia (Rocha et al., 2010). Vermicompost could be an inductor to ATP production also and therefore minimize the harmful effects of waterlogging in *M. oliefera* plants. (2) Waterlogging induces hypoxic soils and therefore death of microbial populations; vermicompost increases microbial populations. (3) Vermicompost increases the water holding capacity of soils. It could be a factor to decrease the hypoxic status both in soils and plants. (4) Potassium in vermicompost could be a key factor because of the ability of root cells to maintain the membrane potential and cytosolic K+ homeostasis. This is central to plant performance under waterlogging (Zeng et al., 2014). (5) The vermicompost contain significant quantities of plant growth hormones and humic acids (HA) which act as plant regulators. It has been demonstrated that HA can have a protective effect possibly through a signalling mechanism initiated by chemical and physical interactions between HA and the plant root system (García et al., 2014).

Chlorophyll content results indicate that *Moringa oleifera* plants suffer more stress under waterlogging conditions. Vermicompost and V+PR treatments induced more chlorophyll contents in leaves of *M. oleifera* plants cultivated under water deficit.

Our results showed that the proline content increased significantly under waterlogging conditions. These results suggest that proline accumulation in waterlogged *Moringa oleifera* plants was probably induced by the application of vermicompost and PR. The induction of proline synthesis under waterlogging may mean a decrease of the cytosolic acidity and maintenance of $NADP^+$/NADPH ratios compatible with the metabolism (Hayat et al., 2012). Olgun et al. (2008) found that proline concentration in waterlogged plants was at least four times higher than that in non-waterlogged plants. In this context, Parvin & Karmoker (2013) reported that waterlogging decreased the accumulation of K^+, Ca^{2+}, Mg^{2+}, NO_3^- and PO_4^{3-}, but increased the accumulation of sugar and proline contents. Finally our results showed that vermicompost and PR treatments could confer tolerance to *M. oleifera* plants by increasing the concentration of proline. On the other hand, waterlogging is one of the major abiotic stresses having detrimental effects on crop production. It may develop due to improper irrigation practices, global warming, anthropogenic factors and natural consequences (Bansal & Srivastava 2015). The generation of reactive oxygen species (ROS) is an integral part of many stress situations, including hypoxia-induced pathways and metabolic adjustments during glycolysis, cytoplasmic acidification and declining ATP levels (Kumutha et al., 2009). Plant tissues contain several ROS scavenging enzymes [e.g., catalase (CAT) and peroxidases (PX)] to control the level of ROS and to protect cells under stress conditions, (Wang et al., 2009). Our result showed that the catalase and peroxidase concentrations increased significantly in *Moringa oleifera* plants treated with vermicompost or PR when plants were subjected to waterlogging conditions. Therefore these results suggested that the application of vermicompost and PR in *M. oleifera* favored the synthesis of both enzymes and inhibited the accumulation of ROS, thus protecting the plants from membrane damage and oxidative stress under waterlogging conditions. Similar results were found by Zhang et al. (2009) in two barley genotypes. These authors found that at early stages of a waterlogging treatment, both peroxidase (POD) and catalase (CAT) activities significantly increased in Xuimai 3 (tolerant cultivar).

CONCLUSIONS

Moringa oleifera plants suffered more stress under waterlogging in comparison to water deficit conditions. However, vermicompost was able to minimize the harmful effects of waterlogging. Plants cultivated under waterlogging showed a smaller height and chlorophyll concentrations, and higher proline concentrations. The application of vermicompost and phosphate rock in *M. oleifera* favored the synthesis of both catalase and peroxidase inhibing the accumulation of ROS, and thus protecting the plant from membrane damage and oxidative stress under waterlogging conditions.

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