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Effect of different vermicompost doses and water stress conditions on plant growth and biochemical profile in medicinal plant, *Moringa oleifera* Lam

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Abstract

Aim: The aim of the present study was to evaluate the effect of different vermicompost application rates on growth, protein and chlorophyll content in leaves of *Moringa oleifera* under water stress in a greenhouse experiment.

Methodology: Soil samples and seeds were collected and characterized. Seedlings grown in a greenhouse in polyethylene bags with 4 kg of soil were subjected to water stress. After 90 days, agronomic variables, chlorophyll and protein content and peroxidase activity were estimated for each treatment and each condition in *Moringa oleifera*.

Results: The results showed that under non-water stress conditions, the levels of vermicompost at a fertilization rate of 500 kg N ha⁻¹ resulted in a significant increase in chlorophyll content, as compared to unamended soil. However, under waterlogging conditions, there was a significant difference between control and vermicompost treatments.

Interpretation: The results indicated that addition of vermicompost to soil improved the growth and photosynthetic rate of *Moringa oleifera* under stress-free and water-deficit conditions. However, protein content only increased significantly when vermicompost was added at a fertilizer application rate of 500 kg N ha⁻¹ both under waterlogging and deficit conditions.

Key words: Abiotic stress, *Moringa oleifera*, Vermicompost, Waterlogging

Dose of vermicompost

0 kg N/ha
300 kg N/ha
500 kg N/ha

Organic fertilization

Crop of *Moringa oleifera*

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Introduction

Moringa oleifera Lam. is commonly known as horseradish tree, which belongs to the Moringaceae family. With approximately 13 species, this family is native to India. However, the species has been cultivated throughout the world, specifically in Asia, Latin America, Florida, the Caribbean, and the Pacific Islands (Fahey, 2005). The leaves of *Moringa* are edible and rich in protein with a balanced amino acid profile, including essential amino acids. They contain vitamins, especially A, B, C and E, comprising also a rich array of bioactive secondary metabolites such as glucosinolates, flavonoids and phenolic acids. It has been reported that different parts of this plant have been used in traditional medicine, including treatment of nervous disorders, hypertension and diabetes (Fahey, 2005).

Plants are subjected to both biotic and abiotic stress during growth and development. Water-deficit stress is a phenotypical characteristic that reveals a state of dehydration in plants due to the lack of available water required to keep cell concentrations at an acceptable and healthy level (Hopkins and Hüner, 2009). In agriculture, drought has been described as soil moisture deficit for a certain crop in a defined growing season (UN FAO 2013). Studies have reported yield losses of $\geq 40\%$ by drought (UN Office for Disaster Risk Reduction, 2011). Water-deficit stress affects morphological parameters, like decreased plant height, diminished root length and area, reduced leaf area, less growth and lower yields. Physiological features are also affected, usually found in the form of decreased photosynthesis, chlorophyll content and water use efficiency. An important component of sustainable agriculture is the use of organic fertilizers such as vermicompost. However, Hosseinzadeh *et al.* (2016) reported that the use of organic amendments in soil can increase the production of field crops and can have a positive effect in drought-stressed crops.

The use of organic amendments in soil can improve the production of field crops and positively effect drought-stressed crops. In recent years, the production and application of vermicompost for enhancing crop productivity and improving soil health has increased greatly for agricultural purposes. The use of vermicompost can increase nitrogen in the soil by about 42%, phosphorus by 29% and potassium by 57% (Sharma and Banik 2014). Vermicompost is highly porous and has an elevated water storage capacity, allowing high ventilation and good drainage (Hosseinzadeh *et al.*, 2015). Addition of organic matter to the soil increases the availability of pores, which is needed to increase air circulation for the growth of plants and microorganisms (Rasool *et al.*, 2007). An important component of sustainable agriculture is the use of organic fertilizers to mitigate water stress, as is the case with vermicompost. Some of the important factors in the process of mitigating water stress have been the rates of nitrogen fertilization of organic amendments that are added to the cultivated soils (Hosseinzadeh *et al.*, 2018). However, Hosseinzadeh *et al.* (2016) reported that

the use of organic amendments in soil can increase the production of field crops and can have a positive effect on drought-stressed crops. Guzmán-Albores *et al.* (2019) reported that the interaction of phosphoric and vermicompost rock cause significant differences in morphometric variables as compared to control. However, no studies on the impact of different rates of vermicompost fertilization in *Moringa oleifera* under water stress conditions is meagre. Thus, in view of the above, the aim of this study was to evaluate the effect of application of different fertilization rates (via vermicompost application) and nitrogen fertilizer rates on growth. Also, possible mitigation of the consequence of water stress in crops of *M. oleifera* was studied.

Materials and Methods

Site description and soil sampling: Soil samples were collected from plots located at the Technological Institute of Tuxtla Gutierrez, Chiapas (México). The experimental site is in the Central Depression of Chiapas, presenting mountainous relief both towards the southern and northern regions. The predominant climate is warm sub-humid with rain during summers.

Characterization of vermicompost and soil: The Characterization of soil samples and vermicompost was done by following the method of Gee and Bauder (1986); Rhoades *et al.* (1989); Bremner (1996). The following parameters were studied moisture, water holding capacity (WHC), texture, pH, electrical conductivity, ion exchange capacity, organic carbon, phosphorus and total nitrogen (Thomas, 1996).

Experimental setup: Seeds collected from "La Escondida" ranch in Chiapas (Mexico) were grown in a green house in polyethylene bags containing 4 kg of soil. Seedlings were watered with tap water at two-day interval. The experiment was carried out in three randomized block design. Treatments were as follows: soil+plant (control); soil+plant+urea (300 kg N ha^{-1}); soil+plant+urea (500 kg N ha^{-1}); soil+ vermicompost (300 kg N ha^{-1}) and soil+vermicompost (500 kg N ha^{-1}). Also, three different levels of water holding capacity (WHC) were used. These were set at 20 % (water deficit), 40 % (optimal conditions) and 60 % (excess water). All the plants were harvested 90 days after germination.

Preparation of sample: For each treatment, fresh leaves 0.5 g of *M. oleifera* were weighed on PA214 Ohaus scale. Samples were then crushed with liquid nitrogen, to make fine powder (100 mesh) and then placed in 1.5 ml tubes and stored at 4°C until further analysis (Chen *et al.*, 2012).

Determination of morphometric variables: Plant length was measured with a Pretul® flexometer while plant diameter was measured with a Surtex® Vernier.

Protein extraction and quantification: Protein extraction from *M. oleifera* leaves was performed following the modified protocol of Chen *et al.* (2012). The extraction buffer contained 0.1 M Tris-

HCl +0.1% ascorbic acid+10% glycerine+1% polyvinylpyrrolidone+5% β -mercaptoethanol at pH 8. A buffer volume of 1 ml was added to each sample and incubated at 4°C for 4 hr. Subsequently, the samples were centrifuged at 15,000 rpm for 15 min at 4°C in a Hermle Z326K centrifuge (Labortechnik GmbH, Stuttgart, Germany). Finally, 1 ml of supernatant was used for protein quantification by the Bradford method (Bradford, 1976), while 10 μ l of the sample was preserved for electrophoresis.

Estimation of peroxidase activity: Peroxidase activity was determined at 90 days by measuring the appearance of tetraguaiacol at 470 nm. The reaction mixture contained 3 ml of extraction buffer, 0.25% (v / v) in 10 mM guaiacol sodium phosphate (pH 6.0) with 10 mM hydrogen peroxide and 100 μ l of enzyme extract. The enzyme activity was calculated by measuring the ratio at 470 nm / min = 0.01, which is defined as 1 unit of enzymatic activity. The specific activity was expressed as IU mg⁻¹ protein (Hammerschmidt *et al.*, 1982).

Estimation of chlorophyll content: The chlorophyll content was determined in the apical leaves using a Konica Minolta Spad 502 computer 90 days after germination. Units were reported as SPAD (Coste, 2010). Six replicates of different leaves were determined as per experimental unit.

Statistical Analyses: A multivariate analysis of variance (ANOVA) was carried out with Tukey test using Statgraphics Centurion XVI software analysis. Samples were considered statistically different below $p < 0.05$.

Results and Discussion

The physico-chemical properties of vermicompost are as follows: pH 8.18; organic carbon content 163 g kg⁻¹; total nitrogen content 11.4 g kg⁻¹; phosphorus content 357.34 mg kg⁻¹; cation exchange capacity 36.59 Cmol g⁻¹; water holding capacity 920 and EC 5.37 dS m⁻¹ respectively. The soil was sandy loam and alkaline (pH of 8.4). Phosphorus content was 11.79 mg kg⁻¹; organic carbon content 6.07 g kg⁻¹; cation exchange capacity 27.61 Cmol g⁻¹; total nitrogen content 0.9 g kg⁻¹; water holding capacity 530 and EC <1 dS m⁻¹. Moreover, these values were similar to the values reported by Ruiz-Valdiviezo *et al.* (2010) regarding physico-chemical characteristics of different soils in the state of Chiapas. Also, these results indicate that these soils are suitable for aiding different types of crops.

The C/N ratio has been used previously as an indicator of fertility and soil productivity. This ratio was higher in soils that have not been cultivated or plowed. Changes in the C/N ratio are attributed to climate change (Ostrowska and Porebska, 2015) due to interaction with microorganisms. However, ratio values lower than 10 for arable soils are associated with high levels of nitrogen, mainly due to excessive use of fertilizers (Ostrowska and Porebska, 2015). This result indicates that biomass in soil organic matter is not enough for nitrogen mineralization, which

generates leachates that can contaminate the soil. Thus, addition of vermicompost favored the ratio, which was higher as compared to native soil (6.7). C/N ratio is considered as one of the most important factors because it is directly related to the necessary nutrients required for microbial activities. A C/N ratio lower than 20 indicates an advanced degree of stabilization of organic matter and a satisfactory degree of maturity of vermicompost. This is suitable for soil amendment (Khawairakpam and Bhargava, 2009) and is necessary because plants cannot assimilate nitrogen unless C/N ratio is less than 20. Moreover, there is substantial evidence in literature suggesting that the use of organic amendment increases soil organic matter (Annabi *et al.*, 2011). In turn, an increase in soil organic matter improves soil quality by increasing soil aggregate stability and other physical properties like water holding capacity, soil porosity, water infiltration and percolation (Leroy *et al.*, 2008).

Growth parameters were evaluated at 90 days. Plants amended with vermicompost at a fertilization rate of 300 kg N ha⁻¹ (V300) attained a height and a diameter of 224.5 cm and 14.11 mm, respectively, as compared to their respective controls which attained 212 cm height and 12.54 diameter. On the other hand, the weight of leaf, stem, root and total weight were not significantly different between treatments (Table 1). In this study, the soil amended with vermicompost improved the growth of *M. oleifera*. These results may be due to an increase in soil fertility level in the amended soils as evidenced by more readily available N in the soil (Roy *et al.*, 2010). Atiyeh *et al.* (2002) also observed that vermicompost contain plant growth regulating materials, such as humic acids and plant growth regulators, such as auxins, gibberellins and cytokinins. These materials are responsible for increasing plant growth and yield in many crops. This is achieved by increasing the activity of microorganisms such as solubilizing bacteria and mycorrhizal fungi, which degrade different components of soil organic matter (Arancon *et al.*, 2005). Numerous studies have reported that the application of organic residues contributed towards increased plant height of okra (Attarde *et al.*, 2012). On the other hand, Mendieta-Araica *et al.* (2013) reported that applying fertilizer at 521 kg ha⁻¹ year⁻¹ increased total dry matter yield.

Protein and chlorophyll content were evaluated at 90 days in all treatments. The highest protein content was obtained with vermicompost at a fertilization rate of 500 kg N ha⁻¹ as compared to the control ($p < 0.05$) (Table 1). There was a significant difference between the treatment soil, V300 and V500, compared to chemical fertilization rate (U300 and U500). In this study, the highest chlorophyll content was observed with V500 treatment. Further, the results revealed that the use of vermicompost increased protein content in *Moringa* leaves, which may be due to high content of humic acids, trace elements, and other important substances that are prevalent in the vermicompost (Lv and Ma, 2005). Additionally, adequate levels of NO₃⁻ (derived from appropriate nitrogen fertilization) increase the amount and activity of nitrate reductase enzyme. In turn, this can lead to a corresponding increase in the reduction potential of NO₃⁻.

Table 1: Effect of fertilization rate of vermicompost on morphometric characteristics and chlorophyll content

Treatments	Height (cm)	Diameter of stem (cm)	Root height (cm)	Weight of leaf (g)	Weight of stem (g)	Weight of root (g)	Total weight (g)	Protein (mg g ⁻¹)	Chlorophyll (unit SPAD)
Soil (control)	212 ^{ab*}	12.54 ^c	30.88 ^a	30.27 ^a	173.45 ^a	88.20 ^a	298.76 ^a	1.42 ^b	27.16 ^a
V500	219.44 ^{ab}	14.41 ^a	32.22 ^a	34.39 ^a	194.81 ^a	90.41 ^a	348.59 ^a	1.53 ^a	29.81 ^a
V300	224.56 ^a	14.11 ^{ab}	34.88 ^a	35.78 ^a	222.30 ^a	94.14 ^a	379.09 ^a	1.46 ^{ab}	28.16 ^a
U500	201.66 ^b	12.81 ^{bc}	31.23 ^a	24.5 ^a	158.45 ^a	82.83 ^a	283.747 ^a	1.50 ^{ab}	20.63 ^b
U300	210.55 ^{ab}	13.09 ^{abc}	32.55 ^a	36.47 ^a	208.48 ^a	92.53 ^a	363.59 ^a	1.46 ^{ab}	22.61 ^b
DMS	22.59	1.5	5.52	17.23	67.29	33.29	105.23	0.09	2.73

*Different letters indicate significant statistical difference ($p < 0.05$); V300 = soil fertilized with vermicompost (300 kg N ha⁻¹), V500 = soil fertilized with vermicompost (500 kg N ha⁻¹); U300 = soil fertilized with urea (300 kg N ha⁻¹), U500 = soil fertilized with urea (500 kg N ha⁻¹)

Table 2: Effects of vermicompost on morphometric characteristics and protein content in leaves of *Moringa oleifera*

Treatments	Plant Height (cm)	Diameter of stem (cm)	Root height (cm)	Weight of leaf (g)	Weight of stem (g)	Weight of root (g)	Total weight (g)	Protein (mg g ⁻¹)
WHC 40 % (Control)								
Soil (control)	252.66 ^{a*}	15.4 ^{ab}	34 ^a	41.3 ^a	249.40 ^a	118.63 ^a	446.67 ^a	1.42 ^c
V500	239.33 ^a	15.92 ^{ab}	32.66 ^a	38.24 ^a	258 ^a	129.64	459.85 ^a	1.66 ^a
V300	252.33 ^a	16.84 ^a	34 ^a	55.32 ^a	305.49 ^a	150.49 ^a	555.55 ^a	1.45 ^c
U500	258 ^a	14.47 ^{ab}	31.66 ^a	31.86 ^a	250.2 ^a	152.86 ^a	493.34 ^a	1.44 ^c
U300	241.33 ^a	13.93 ^b	32.33 ^a	50.13 ^a	281.53 ^a	138.41 ^a	502.87 ^a	1.52 ^b
WHC 60 % (Waterlogging)								
Soil (control)	216 ^{ab}	12.76 ^a	31.66 ^a	37.83 ^{ab}	194.98 ^a	99.18 ^a	361.97 ^a	1.54 ^{ab}
V500	229.66 ^{ab}	15.41 ^a	34.33 ^a	49.28 ^a	213.22 ^a	94.46 ^a	395.40 ^a	1.55 ^a
V300	236.33 ^a	14.56 ^a	37.33 ^a	37.23 ^{ab}	248.7 ^a	98.6 ^a	400.35 ^a	1.41 ^c
U500	203.66 ^b	14.27 ^a	37.04 ^a	26.94 ^b	160.42 ^a	72.11 ^a	293.23 ^a	1.48 ^b
U300	225.66 ^{ab}	14.73 ^a	38.66 ^a	36.8 ^{ab}	237.53 ^a	99.42 ^a	400.48 ^a	1.48 ^b
WHC 20 % (Water deficit)								
Soil (control)	167.33 ^{ab}	9.48 ^b	27 ^a	11.69 ^a	75.95 ^a	46.81 ^a	88.25 ^{ab}	1.42 ^b
V500	189.33 ^a	11.90 ^a	29.66 ^a	15.66 ^a	113.22 ^a	47.13 ^a	188.93 ^a	1.59 ^a
V300	185 ^a	10.93 ^{ab}	33.33 ^a	14.79 ^a	112.73 ^a	39.76 ^a	173.24 ^a	1.40 ^b
U500	143.33 ^b	9.69 ^b	25 ^a	14.69 ^a	64.75 ^a	23.53 ^a	64.75 ^b	1.40 ^b
U300	164.66 ^{ab}	10.61 ^{ab}	26.66 ^a	22.49 ^a	106.38 ^a	39.76 ^a	187 ^a	1.38 ^b

*Different letters indicate significant statistical difference ($p < 0.05$); V300 = soil fertilized with vermicompost (300 kg N ha⁻¹), V500 = soil fertilized with vermicompost (500 kg N ha⁻¹); U300 = soil fertilized with urea (300 kg N ha⁻¹), U500 = soil fertilized with urea (500 kg N ha⁻¹)

, thereby providing a greater overall capacity for the synthesis of amino acids and proteins, or assimilation of nitrogen (Friedman, 2004). Organic fertilizers such as vermicompost boost the content of soil nutrient elements, which increase the availability of these elements for plants, ultimately leading to enhanced chlorophyll content (Tadayyon *et al.*, 2018). Nardia *et al.* (2002) reported that the application of vermicompost increased the chlorophyll content due to the presence of abundant amount of humic substances.

The results showed that water deficit and waterlogging (20 % and 60% WHC) significantly decreased the morphological features when compared with their respective control (Table 2). These results showed that under severe stress and waterlogging

conditions (20 % and 60% WHC), vermicompost treatments at 300 and 500 kg N ha⁻¹ allowed for a significant increase in plant height and stem diameter compared to the control and chemical fertilization (U300 and U 500). Under no-stress conditions (40 % WHC), vermicompost at 300 kg N ha⁻¹ allowed a significant increase of stem diameter compared to chemical fertilization (Table 2). On the other hand, no significant statistical difference ($p < 0.05$) were reported for root length, weight of fresh leaves, stems, and roots under different growing conditions. However, comparison of the interaction between vermicompost and water deficit on total weight, demonstrates thos of vermicompost at 300 and 500 kg N ha⁻¹ increased the total weight of the plant as compared to chemical fertilization applied at 500 kg N ha⁻¹ (Table 2).

There was a significant difference in protein content between vermicompost treatments and control. Under non-stress conditions, the vermicompost (V500) treatments showed an increase in protein content over that of the control and chemical fertilization (U300 and U500). At low water deficit and waterlogging conditions, 500 kg N ha⁻¹ vermicompost showed a significant increase in protein content over that of the control and chemical fertilization (Table 2). Several studies have shown that plant height is reduced under water stress conditions (Ganjeali *et al.*, 2011). Bowden *et al.* (2010) reported that an increase in plant height and number of pods will result from the stimulation of auxin-like substances produced during vermicompost consumption. Humic and fulvic acids and other organic acids found in vermicompost (Hosseinzadeh *et al.*, 2015), as well as the frequency of nutrients, (especially N), can stimulate plant growth (Amiri *et al.*, 2017).

The positive effect of soil amendment with vermicompost on plant height and number of pods was observed in *Cicer* and *Pisum* (Sinha *et al.*, 2010). Moreover, Mirhashemi *et al.* (2009) evaluated the effects of different levels of manure on ajwain (*Carum copticum*) and fenugreek (*Trigonella foenum*) plants. They also found that by increasing the use of this organic fertilizer, the crop growth rate also increased. Addition of vermicompost to the soil increases availability of nutrients for the plant. By improving the physical conditions of the soil, an appropriate bed for root development was created, also causing an increase in the shoot growth (Arancon *et al.*, 2004). An important feature of soil amendment with vermicompost is the increase of some soil microbial population such as nitrogen stabilizers, actinomycetes, fungi spores, and mycorrhizal symbiosis with the roots of plants (Hosseinzadeh *et al.*, 2015). Nitrogenous heterocycles are raw materials required for protein biosynthesis. Thus, an increase in protein content in leaves is directly related to enhanced nitrogen uptake by the plants (Hosseinzadeh *et al.*, 2016).

The results of this study suggest that under non-water stress conditions, vermicompost at 500 kg N ha⁻¹ resulted in a significant increase in chlorophyll as compared to control (soil). However, under waterlogging, there was a significant difference between control and vermicompost treatments (Table 3). Under water deficit conditions, the addition of vermicompost at 500 kg N ha⁻¹ resulted in a significant increase in chlorophyll content as compared with chemical fertilization (U300 and U500). Hosseinzadeh *et al.* (2018) reported that chlorophyll content is known as tolerance index to water stress in plants. The results of the study showed that addition of vermicompost to soil influenced water deficit conditions by increasing the content of macronutrients. Addition of macronutrients could in turn activate enzymes involved in chlorophyll synthesis and plant. In addition, the high content of nitrogen and its metabolism might be closely related to carbon assimilation in photosynthesis, resulting in extremely important roles in regulating plant growth and development (Ibrahim and Jaafar, 2011).

Peroxidase activity in *M. oleifera* leaf extracts was significantly affected by the stress conditions of the crop. POX

Table 3: Effects of vermicompost on the content of chlorophyll in leaves of *Moringa oleifera*

Treatments	Chlorophyll (Unit SPAD)
WHC 40 % (Control)	
Soil (control)	25.42 ^{ab}
V500	32.37 ^a
V300	29.37 ^{ab}
U500	22.27 ^b
U300	20.57 ^b
WHC 60 % (Waterlogging)	
Soil (control)	35.08 ^a
V500	30.23 ^b
V300	28.08 ^b
U500	23.46 ^c
U300	23.86 ^c
WHC 20 % (Water deficit)	
Soil (control)	24.08 ^a
V500	26.83 ^a
V300	25.83 ^a
U500	16.17 ^b
U300	17.10 ^b

*Different letters indicate significant statistical difference ($p < 0.05$); V300 = soil fertilized with vermicompost (300 kg N ha⁻¹), V500 = soil fertilized with vermicompost (500 kg N ha⁻¹); U300 = soil fertilized with urea (300 kg N ha⁻¹), U500 = soil fertilized with urea (500 kg N ha⁻¹)

Table 4: Peroxidase activity in leaves of *Moringa oleifera* under water stress

CRA	Activity of peroxidase (UEA mg ⁻¹ of protein)
WHC 60 % (Waterlogging)	76.76 ^{ab}
WHC 40 % (Control)	56.60 ^b
WHC 20 % (Water deficit)	59.15 ^b
DMS	7.77

*Different letters indicate significant statistical difference ($p < 0.05$)

activity was increased to 76.66 units (UEA mg⁻¹ of protein) under waterlogging conditions (60 WHC) as compared with the control (40 % WHC) (Table 4). However, in water deficit conditions (20% WHC), no significant difference was found ($p < 0.05$) compared to the control. These results could be due the production of reactive oxygen species (Vurukonda *et al.*, 2016), which is another major factor that impairs plant growth under water deficit stress (Wei *et al.*, 2015). Shi *et al.* (2007) reported that plants employ numerous defense systems to combat the destructive effects of drought and waterlogging. These systems include the development of antioxidants and enzymes, which is a major mechanism that enables plants to control ROS (Borella *et al.*, 2017). Therefore, positive correlations have been reported between the activities of different antioxidant enzymes with crop resistance to stress (Caverzan *et al.*, 2016). In this study, water deficit and

waterlogging stimulated the activity of POX. Consequently, these findings show that the activation of antioxidant enzyme is an important element pertaining to the responses of *Moringa oleifera* under water stress conditions.

The application of vermicompost significantly improved photosynthetic and physiological parameters under non-stress (40 % WHC) and water deficit (20 % WHC) conditions, compared with the chemical fertilization. Also, the advantages of amending soil with vermicompost on water stress plants were verified.

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