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Foliar Application of Moringa Leaf Extracts Affects Growth, Yield and Mineral Composition of Pepper (*Capsicum Annuum* L.) under Greenhouse Conditions

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ABSTRACT

The higher cost and increased pollution caused by the intensive use of fertilizers in growing vegetables necessitates the use of safer organic bio-stimulants to partially substitute fertilizers. Analyses of leaf extracts of *Moringa oleifera* and *Moringa peregrina* in the present study confirmed their rich content of diverse compounds and elements and indicated that except for Mg and Ca, *M. oleifera* outyielded *M. peregrina* for the measured elements and bio-constituents. Foliar spray of leaf extracts of *M. oleifera* and *M. peregrina* at (1:10, 1:20, 1:30 extract: distilled water) was attempted on pepper plants under greenhouse conditions every two weeks for five times starting 30 days after transplanting date. Enhanced vegetative growth parameters (plant height, number of branches and leaves, plant fresh and dry weights, leaf area, and leaf chlorophyll-content) and better yield attributes (Fruit yield·plant^{-1} and hectare^{-1}) were obtained by foliar spraying of plants with the concentrated extract 1:10 of moringa species. The vegetative growth of pepper plants was significantly influenced by extract concentration, moringa type·concentration, but not for moringa type ($P \leq 0.05$). However, moringa type, extract concentration and their interactions significantly affected pepper fruit-yield·plant^{-1}, pepper fruit-yield·ha^{-1} and mineral content of pepper leaves ($P \leq 0.05$). Irrespective of moringa type, the highest extract concentration 1:10 resulted in the highest yield ·plant^{-1} (1.68 kg) and yield·hectare^{-1} (16.88 ton) of peppers. The present study highlighted the potential of using extracts of moringa trees in organic farming.

Keywords: Foliar application, leaf extracts, *Capsicum Annuum* L., *Moringa oleifera*, *Moringa peregrina*.

INTRODUCTION

Sweet pepper, known as *Capsicum annuum* L., is an important culinary vegetable. Pepper fruits are also consumed fresh or included in salad. Worldwide, it ranks second after tomato for commercial export and in local consumption because it is used in many food industrial preparations (Gobie, 2019). Its fruit contains important compounds such as proteins, vitamins as A, B1 and C, high contents of antioxidant compounds, e.g. capsaicin and capsanthin, in addition to appreciable amonts of minerals (Olaniyi and Ojetayo, 2010, Aminifard et al., 2012, Howard et al., 2000). Therefore, this crop combines both nutritional and medicinal benefits.

Jordan valley and highlands are the principal cropping area of this important vegetable in Jordan. Besides supporting the local need, there is an export of Jordanian vegetables including sweet and hot peppers to the Gulf region and other countries which plays a role in increasing Jordan's national income (Leeters and Rikken, 2016). Factors as the increased population, fragmentation of land have caused a dramatic decrease in the area available for agriculture. This necessitates the adoption of new strategies that aims at more efficient land and resources use. For this reason, off-season cultivation of peppers and other highly consumed crops is recently practiced (Sukmawati and Dasipah, 2020).

Intense cultivation of vegetables requires the use of more fertilizers and increases the cost of

production (Ganjare et al., 2013). This not only can be a burden on farmers but also might resulted on the long run in soil degradation as well as increased pollution of the environment including contamination of the ground water (Lan and Xia, 2008; Valentin et al., 2017; Bijay-Singh and Craswell, 2021). Therefore, there is a need to seek more environmentally safe plant growth regulators (PGPs).

The use of proper PGRs along with other nutrient-complements can result in enhanced plant growth which in return caused in many instances increased plant tolerance to stresses (Ashraf and Foolad, 2007; Farooq et al., 2009). The promoting effects of cytokinis, a group of PGRs, on plant growth and biochemical constitutes, and on delaying senescence is well documented (Wu et al., 2021). However, these hormonal substances are highly expensive and impractical to use in large-scale plantations. Natural plant growth-stimulating extracts as that of *M. oleifera* and *M. peregrina* leaf extracts (MLE) is therefore highly recommended. These substances are easy to use, environmentally safe and inexpensive (Mashamaite et al., 2022).

Moringa, which includes plants that ranges from tiny herbs to massive trees, is known for its rich constituents of vitamins, e.g. vtamin C, antioxidants, minerals and PGRs, namely cytokinins (Makkar et al., 2007, Gopalakrishnan et al., 2016). Its leaves can be used fresh, or dried and prepared extracts can be foliar applied on plants (Olson, 2001, Batool et al., 2020). Besides, its immature pods, roots or leaves can be consumed as a vegetable (Ebert and Palada, 2015). Foliar application of its leaf-extract on target plants is supposed to provoke cytokinin synthesis, thus causing a variety of physiological responses that might include increased leaf area and photosynthetic activity and consequently counteracting premature leaf senescence (Mashamaite et al., 2022). Therefore, the objective of the current study was to evaluate the effects of foliar application of different concentrations of *M. oleifera* and *M. peregrina* leaf extracts on growth, yield and leaf mineral content of sweet peppers under greenhouse conditions.

MATERIALS AND METHODS

The study was conducted at a greenhouse located in the National Agricultural Research Center (NARC) at Al-Shoubak/ Jordan.

Preparation and analysis of moringa leaf extract (MLE)

Full-grown young trees raised at Ghor Al-Safi Agricultural Research station, Al Karak served as the source of leaf samples of M. *oleifera* and M. *peregrina*. The leaves were washed, then left to dry at room temperature for 14 days and finally powdered using electrical miller. The extracts were constituted by adding 675 ml of 80% aq. Ethanol to twenty grams of the milled leaves of the two types of moringa (Makkar and Becker, 1996). The extracts were diluted as follows (extract: distilled water): 1:30, 1:20, 1:10 to obtain the assigned concentrations. Control consisted of (distilled water; control 1) and (ethanol 80%, control 2). The atomic absorption method was used to determine the nutrient content of the air-dried leaves of *M. oleifera* and *M. peregrina*. Zeatin content was quantified using High-Performance Liquid Chromatography (HPLC). The samples were compared against a standard stored solution of zeatin. The chromatographic peaks of the samples were determined using the methodology described by Ortiz and Florez, 2008.

Experimental design

A split plot design with three replicates was employed. Main plots consisted of type of moringa leaf extracts (MLE) (*M. oleifera* and *Moringa peregrina*), whereas subplots were the five concentrations of each type {1:30, 1:20 and 1:10, control 1 (distil water), control 2 (ethanol 80%). The first spraying started thirty days after transplanting by giving each plant 25 ml of each concentration at two-week interval and each treatment was repeated five times.

Growth parameters

Growth parameters including plant height, number of branches and leaves were measured every two weeks along the growing season. At the end of the experiment: fruit yield per plant and hectare in addition to the fresh and dry weight of shoots and roots were measured during harvesting period. Plant samples were dried in an oven to a constant weight at 75 °C for 72 hours. Leaf area (cm^2) was measured on four samples per treatment at the end of the growing season using the photoelectrical method (Cox, 1972).

Leaf biochemical parameters

SPAD Chlorophyll Meter from Minolta (Model 502) was used to measure leaf chlorophyll content using samples collected every two weeks from each treatment. Leaf samples were collected at the end of the growing season, then dried in an oven at 68 °C for 72 hours and finally were grounded. Energy Dispersive X-Ray Fluorescence Spectrophotometer (EDX-7000) located at Al Hussein Bin Talal University was used for the determination of Fe, Zn, Ca, P and K mineral contents. Total N was analyzed by Kjeldhl digestion method.

Data analysis

The collected data were subjected to analyses of variance (ANOVA) using Statistical Analysis System (SAS) at 5% probability level. The significant deferences between the means was compared by the Fisher Least Significant Difference (LSD) (Gomez and Gomez, 1998).

RESULTS AND DISCUSSION

Chemical- and bio-constituents of *M. oleifera* and *M. peregrina* leaves

Figure 1A and B show chromatographic peaks of zeatin in moringa leaves of both species as determined by HPLC. Analyses indicated a high content of zeatin in leaves of *M. oleifera* (2395.8 $\mu g \cdot g^{-1}$) and *M. peregrina* (728.9 $\mu g \cdot g^{-1}$). The mineral contents of *M. oleifera* and *M. peregrina* leaves analyzed in the present study were: 1.22 and 1.11% for K, 1.99 and 2.51% for Ca, 0.31 and 0.27% for P, 0.77 and 1.34% for Mg, 0.35 and 0.18% for Cl, respectively (Fig. 2A). Additionally, leaves of *M. oleifera* and *M. peregrina* contained other elements: 494 and 464 $\mu g \cdot g^{-1}$ for Na, 286 and 219 $\mu g \cdot g^{-1}$ for Fe, 32.1 and 24.7 $\mu g \cdot g^{-1}$ for Mn, 6.25, 2.43 $\mu g \cdot g^{-1}$ for Cu, and 30.4 and 25.13 $\mu g \cdot g^{-1}$ for Zn (Fig. 2B). Protein content of both species was also analyses (21.36% for *M. oleifera* and 6.54% for *M. peregrina*). Thus, results of the present study indicated that moringa genus is a rich source of minerals and growth regulators such as cytokinin's (Fig. 1A & B, Fig. 2A & B). Leaf extracts prepared from moringa spps were reported to be a rich source of hormones as zeatin, gibberellins and indole-3-acetic acid as well as micro- and macro elements (Rady and Mohamed, 2015). Except for Mg and Ca, *M. oleifera* outyielded *M. peregrina* for the measured elements and bio-constituents as indicated by the current results. Al-Rawashdeh et al. (2016) reported relatively high Mg content in leaves of *M. peregrina*. Earlier reports indicated that moringa leaves are a rich source of nutrients such as vitamin A and C, protein, calcium, and

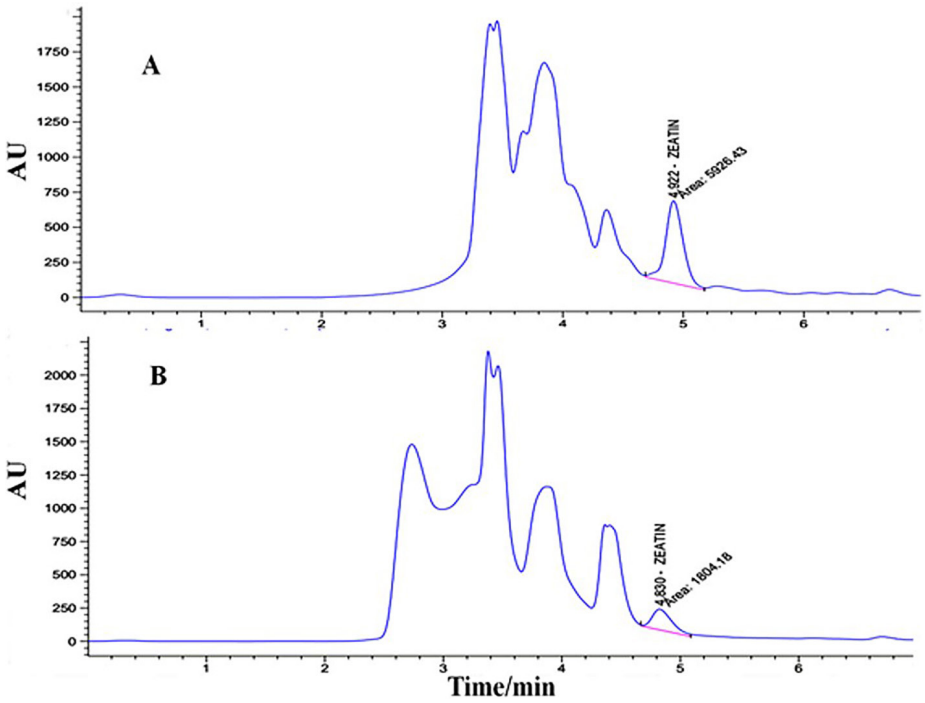

Figure 1. Chromatgraphic peaks used to determine content of zeatin in leaf samples of *M. oleifera* (A) and *M. peregrina* (B)

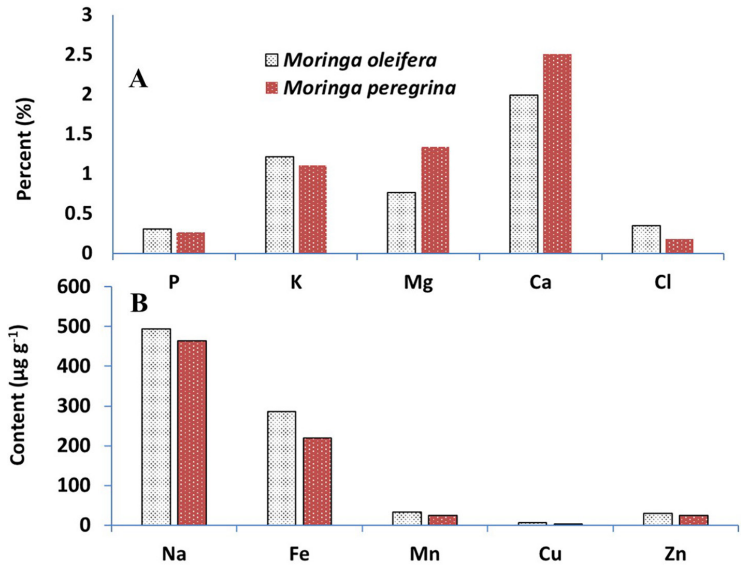

Figure 2. Content of minerals in leaf samples of *M. oleifera* and *M. peregrina*

potassium in comparison to traditional sources of food containing these constituents and, thus, are added to food preparations and used as a fodder for animals (Nouman et al., 2012; Abd Rani et al., 2018). Besides, the high content of zeatin in moringa leaves in the present study explains the boosting role of extracts of moringa leaves on pepper plant growth under greenhouse conditions and in reported *in vitro* culture studies (Hoque et al., 2022; Nihayati and Najah, 2021).

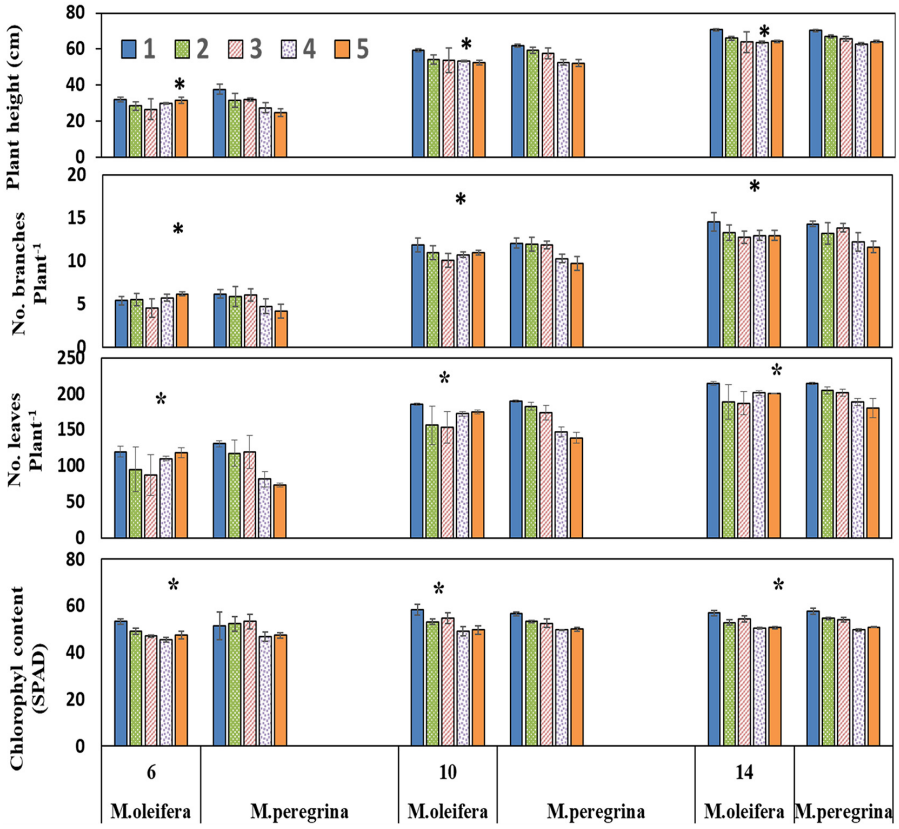

Figure 3. Effects of leaf extract concentrations of *M. oleifera* and *M. peregrina* on plant height, number of branches and number of leaves measured every four weeks starting the 6th week of growth in greenhouse along the growing season. Treatments consisted of moringa leaf extracts diluted by combining in distilled water at concentrations of: 1:10 (trt 1); 1:20 (trt2); 1:30 (trt3), control 2 (ethanol 80%, trt4); control 1 (distilled water, trt 5). * symbolizes significant interactive effects between moringa type and extract concentration at 0.05 level

Growth parameters of pepper

The use of the least dilution of leaf extracts of both Moringa species (1:10) resulted in enhanced plant height, no. of branches and leaves plant^{-1}, and chlorophyl content of peppers (Figure 3). All parameters, except chlorophyl content, were higher at week 6 after transplanting in response to (1:10) dilution of *M. peregrina* leaf extracts compared to other treatments. Thereafter all parameters increased progressively on weeks 10 and up to week 14 following transplanting and were overall the highest in response to (1:10) dilution of *M. peregrina* leaf extracts than other treatments. *Moringa oleifera* leaf extracts gave variable results at week 6 on pepper growth; using (1:10) dilution only enhanced chlorophyl contents significantly among the studied parameters compared to the other dilutions. However, from week10 and up to week 14, 1:10 dilution of *M. oleifera* leaf extracts have induced the highest response of all pepper growth parameters compared to other dilutions. The highest response of peppers: height of plants (70.4 cm), no. of branches and leaves plant^{-1} (14.4 and 214.3, respectively), and chlorophyl content (57.2 SPAD) were obtained using the concentrated extracts (1:10) irrespective of the used moringa species. Results indicated no differences between the two Moringa types except for plant height which showed favorable effects using (1:10) dilution of *M. peregrina* than *M. oleifera* during the growing season particularly weeks 6 and 10 after transplanting. Leaf extracts of *M. oleifera* and *M. peregrina* applied at 1:10 concentration significantly also produced heavier shoots at end of study (fresh weights: 412.3 and 418 g, dry weights: 81.33 and 82.33 g, respectively) (Figure 4). Likewise, foliar application of 1:10 dilutions of *M. oleifera* and *M. peregrina*, although not significantly different between the two moringa types resulted in the highest root fresh (80.0 and 84.6 g, respectively) and dry weights (46.3 and 39.6g, respectively) among the treatments tested (Figure 4). The leaf area of pepper plants was significantly influenced by foliar application of *M. oleifera* and *M. peregrina* extracts with the highest response being obtained at 1:10 concentration (65.8 and 61.3 cm^2, respectively) (Figure 5). Leaf extracts of both moringa types applied at 1:20 ranked second in terms of their influence on pepper

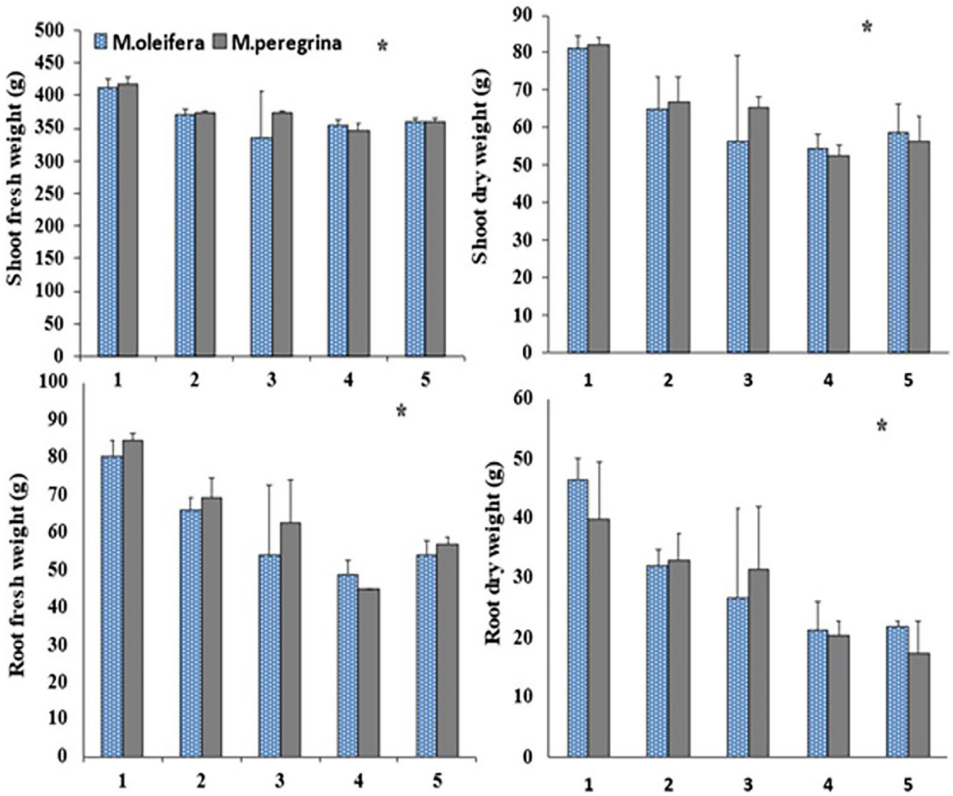

Figure 4. Effects of leaf extract concentrations of *M. oleifera* and *M. peregrina* on fresh and dry weight of shoots and roots at the end of the growing season. Treatments consisted of moringa leaf extracts diluted by combining in distilled water at concentrations of : 1:10 (trt 1); 1:20 (trt2); 1:30 (trt3), control 2 (ethanol 80%, trt4); control 1 (distilled water, trt 5). * symbolizes significant interactive effects between moringa type and extract concentration at 0.05 level

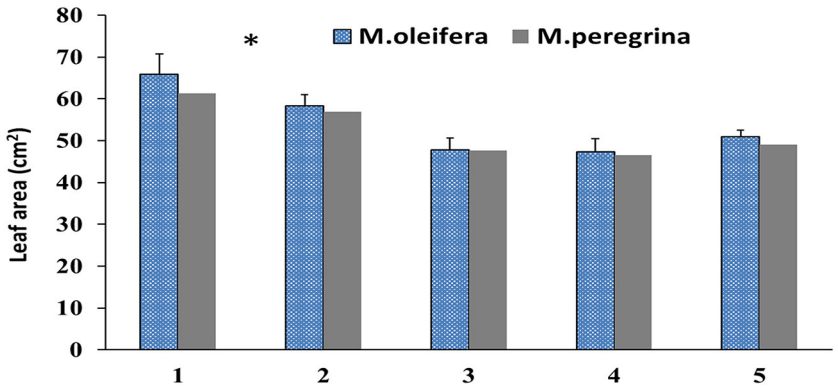

Figure 5. Effects of leaf extract concentrations of *M. oleifera* and *M. peregrina* on leaf area at the end of the growing season. Treatments consisted of moringa leaf extracts diluted by combining in distilled water at concentrations of : 1:10 (trt 1); 1:20 (trt2); 1:30 (trt3), control 2 (ethanol 80%, trt4); control 1 (distilled water, trt 5). * symbolizes significant interactive effects between moringa type and extract concentration at 0.05 level.

Table 1. Interactive effect of moringa type and concentration on pepper leaf mineral content under greenhouse Conditions

Moringa type	Concentration (extract: d. H_2O)	Leaf mineral contents					
		N (%)	P (µg g^{-1})	K (µg g^{-1})	Fe (µg g^{-1})	Ca (µg g^{-1})	Zn (µg g^{-1})
M. oleifera	Control1	2.55 h	2895.11 de	22487.48 bcd	270.71 ab	14722.27 d	59.44 de
	Control 2	2.88 def	2831.10 def	20936.49 de	253.82 c	13711.07 d	59.44 de
	1:30	2.92 de	3077.03 d	22094.11 bcde	247.27 cde	14620.20 d	66.10 ab
	1:20	3.11 ab	3474.18 bc	23414.48 ab	252.99 cd	18399.02 ab	64.52 abcd
	1:10	3.20 a	4100.30 a	25176.05 a	274.91 a	19563.77 a	67.83 a
M. peregrina	Control1	2.53 h	2616.57 fgh	21938.07 bcde	202.06 h	14668.94 d	49.89 gh
	Control 2	2.71 gh	2347.86 h	20329.83 e	211.42 h	13518.86 b	49.05 h
	1:30	2.83 defg	2478.59 h	21716.67 bcde	216.34 gh	15569.92 d	51.06 gh
	1:20	2.95 bcd	2775.01 efg	21862.75 bcde	228.48 fg	14218.49 d	55.08 efg
	1:10	3.09 abc	3646.62 b	22854.24 bc	241.27 cde	18202.81 abc	64.68 abc

Note: * means followed by the same letter along the columns are not significantly different at $p \leq 0.05$.

leaf area, and the least response was obtained using 1:30, control 1, and control 2 (Figure 5). The concentration of the minerals in leaves of peppers was generally higher using the leaf extracts of *M. oleifera* than using that of *M. peregrina* regardless of the leaf extract dilutions (Table 1). The highest concentrations of *M. oleifera* and *M. peregrina* leaf extracts (1:10) significantly resulted in the highest mineral content of peppers leaves compared to the other treatments: 3.20 and 3.09% for N, 4100.3 and 3646.6 µg·g^{-1} for P, 25176.1 and 22854.2 µg·g^{-1} for K, 274.9 and 241.3 µg·g^{-1} for Fe, 19563.7 and 18202.8 µg·g^{-1} for Ca, and 67.8 and 64.7 µg·g^{-1} for Zn, respectively (Table 1).

Fruit yield

The concentrated extracts (1:10) of *M. oleifera* and *M. peregrina* produced the highest number of fruits $plant^{-1}$ (17.7 and 17.9 fruits·$plant^{-1}$, respectively), fruit yield $plant^{-1}$ (1.7 and 1.6 kg, respectively) and fruit yield $area^{-1}$ (17.5 and 16.3 ton·ha^{-1}, respectively) (Figure 6). Increasing leaf extract concentration from 1:30 to 1:10 was overall shown to increase yield $plant^{-1}$ and yield $area^{-1}$. Fruit weight was inversely proportional to extract concentrations in particular when using leaf extracts of *M. oleifera* than *M. peregrina* (Figure 6).

In general, the two moringa species and concentrations prepared from extracts of the current study had significant interactive effects on the growth parameters and fruit yield of sweet pepper (P=0.05). In agreement with the results of the present study several reports indicated the favorable effects of Moringa on various pepper species which included significant effects on plant height, no. of leaves and branches, seedlings fresh and dry weight, leaf chlorophyl content, yield components and mineral content (Abou El-Nour and Ewais,

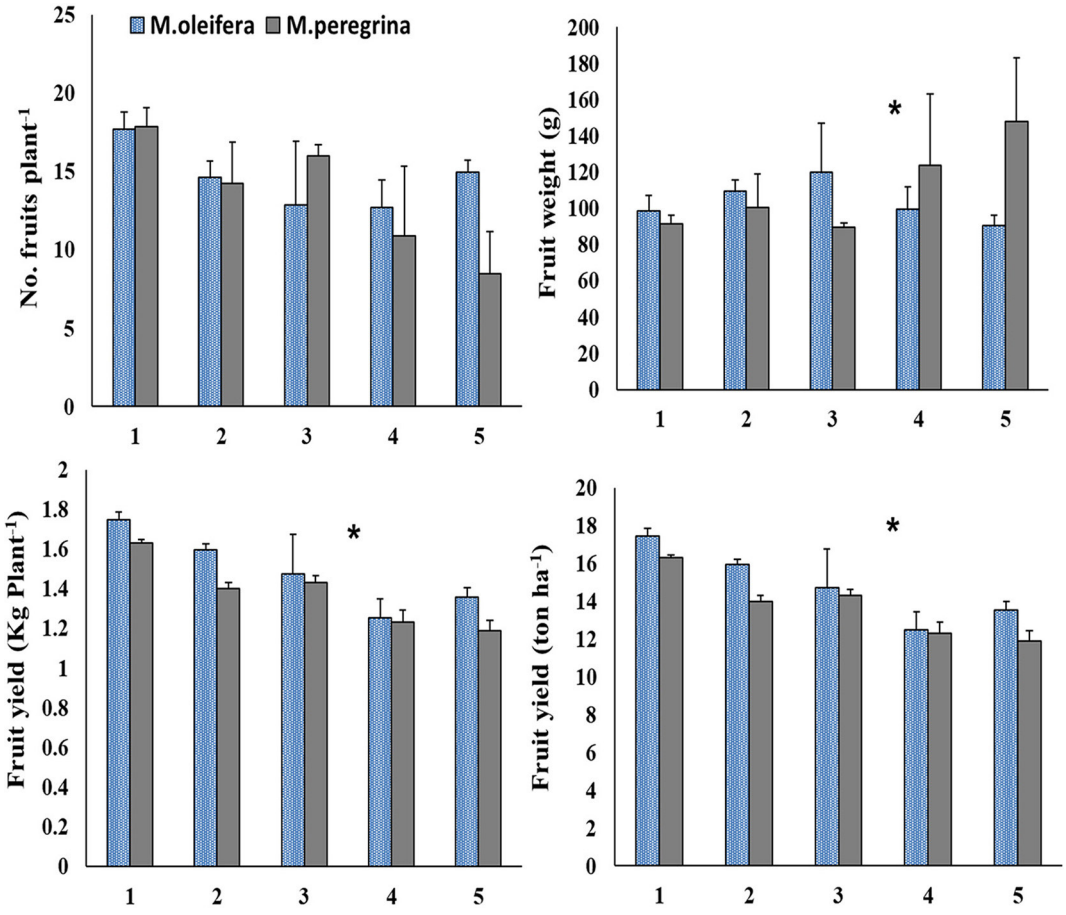

Figure 6. Effects of leaf extract concentrations of *M. oleifera* and *M. peregrina* on fruit yield attributes of peppers at the end of the growing season. Treatments consisted of moringa leaf extracts diluted by combining in distilled water at concentrations of : 1:10 (trt 1); 1:20 (trt2); 1:30 (trt3), control 2 (ethanol 80%, trt4); control 1 (distilled water, trt 5). * symbolizes significant interactive effects between moringa type and extract concentration at 0.05 level.

2017, Dunsin and Odeghe, 2015, Aluko, 2016, Weerasingha and Harris, 2020). The enhanced differentiation of shoots and leaves in pepper plants in response to moringa leaf extracts according to our results might be due their content of hormones, namely cytokinins, that cause higher cell extension which promotes enhanced metabolite production and thus higher food translocation to young expending shoots (Nouman et al., 2012, Rady et al., 2015). Besides, moringa leaf extracts help delay senescence, and can improve the concentration of photosynthetic pigments like chlorophyll a and b as they have high magnesium content which is supported by the present findings (Weerasingha and Harris, 2020). Moringa leaf extract is considered generally a rich source of pigments as xanthin, lutein, beta-carotene and alpha-carotene (Owusu, 2008). The enhanced vegetative growth of peppers in the current study as indicated by above-mentioned parameters together with the higher leaf area of pepper plants recorded in response to moringa leaf extracts, in particular at 1:10 concentration can in principle support the higher observed fresh and dry weights of pepper plants compared to other treatments. The higher growth-promoting hormones of cytokinins as shown by current study and the greater amount of crude proteins found in moringa leaves as reported by Foidl et al. (2001) may be responsible for the increased pepper leaf area which may support in turn higher light interception, and photosynthesis. This in turn may cause more assimilate translocation to leaves and fruits of peppers in particular those treated with concentrated moriga extracts and thus explained among other factors the higher yield. Moringa leaf extracts in agreement with the present results promoted fresh and dry weight relations in pepper cv. California wonder as well in other plants as beans and rocket plant (Abou El-Nour and Ewais, 2017; Mvumi et al., 2012; Mona, 2013).

In conclusion, foliar application of leaf extracts of *M. peregrina* and *M. oleifera* showed positive effects on vegetative growth and yield components of pepper plants grown under greenhouse conditions.

The highest response was obtained using 1:10 concentration of the extract of both moringa species. The findings of the present study highlighted the beneficial effects of moringa extracts which can be further tested for other applications as disease control of peppers and may provide valuable eco-friendly nutrients for plant growth that with the other new natural tested bio-stimulant compounds can partially substitute fertilizer requirements and may be a promising tool in organic farming of vegetables.

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