http://dx.doi.org/10.22161/ijeab/2.5.59 ISSN: 2456-1878

Effect of Organic Nutrition in the Nursery Growth and Nutrimental Content of Native Avocados of Ometepec, Guerrero, Mexico

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***Abstract*— *In Mexico, there are several types of wild and criollo avocados that constitute a genetic heritage of this species; these avocados currently grow in an unordered manner on farmer's lands and in backyards, and they need to be studied as they are being lost because of agricultural activities and edaphoclimatic and phytosanitary factors. On the other hand, in orchards and avocado nurseries, high amounts of chemical fertilizers and pesticides are used affecting the physicochemical and microbiological properties of the soil, modifying the flora and fauna and polluting aquifers and springs that cause health problems among consumers. Therefore, it is very important to have a more friendly agriculture with the nature. The aim of this work was to evaluate under nursery conditions, the effect of organic fertilizers on 12 genotypes (rootstocks) of native avocados of Ometepec, Guerrero, Mexico, under an experimental design of random blocks, with four treatments: T1: sheep manure,T2: Bovine manure, T3: mycorrhizae and T4 (control: water) in four replicates. The variables were: plant height (PH), stem diameter (SD), number of leaves: young (NYL) and mature (NML) per plant; and the content of NO_3^-, K^+, Ca^{2+} and Na^+ ions obtained by petiole extraction, and the chlorophyll content measured with SPAD, in young (CYL) and mature (CML) leaves. Additionally, an analysis of variance and Tukey mean tests ($P \leq 0.01$ and 0.05) and LSD ($P \leq 0.05$) were done. It was found that sheep manure was superior to other treatments in PH (76.7 cm), SD (7.2 mm), NYL (6.5 leaves/plant), NML (18.4 leaves/plant), CML (40.2 SPAD) and Ca^{2+}(1495ppm). In conclusion, the sheep manure was better than the bovine, mycorrhiza and control (water) as it affected positively the behavior of rootstocks in plant height, stem diameter and number of young and mature leaves. In addition, organic nutrition showed no significant response in the chlorophyll content of young and mature leaves. Young leaves only reached 50% of the chlorophyll content compared to mature leaves.*

Keywords*— *Native Avocados, Vegetative Growth of Rootstocks, Organic Nutrition.

I. INTRODUCTION

In conventional agriculture, heavy doses of chemical fertilizers and pesticides are often used to correct nutrient deficiencies in soils and to improve crop yields. However, these chemicals cause health problems among consumers (Larios *et al.*, 2011; Márquez-Quiroz *et al.*, 2014) and to the environment, as in areas where these substances are applied, the leached water draws nitrates, that pollute aquifers and springs in avocado producing regions (Tapia *et al.*, 2012). In this sense, it has been reported that higher concentrations than10 mg L^{-1} of $N\text{-}NO_3$ in drinking water can cause serious diseases in humans and young animals (Killpack and Bucholz, 1993); the nitrogen loss in avocado orchards increases during the raining season, July and October, and they pollute the environment as the leachatecarries out the nutrients. In the same way, irrigation contributes to this loss of chemical fertilizers as every year the traditional irrigation produced 80-96 $mgL^{-1}$of $N\text{-}NO_3$ leachate, while the located irrigation only produced from 36-47 mg L^{-1} (Tapia *et al*., 2012). In addition, these

http://dx.doi.org/10.22161/ijeab/2.5.59 *ISSN: 2456-1878*

chemical fertilizers affect the physicochemical and microbiological properties of soil, as they modify its pH, structure, aeration and porosity, as well as the flora and fauna (Trinidad *et al*., 2015).In the mutualistic associations, known as arbuscular mycorrhiza, the fungus colonizes the root cortex in an extra and intracellular way, developing an intricate external mycelium that surrounds the root of the colonized plants. This mycelium forms a continuous connection between the solution of the soil and the plant, which allows the uptake of ions from the soil and their transport to the root of the host. In an opposite way, the arbuscular mycorrhizalfungus (AMF) receives carbon compounds from photosynthesis of the plant, which are necessary for its metabolism because it is a symbiont, which requires interaction with the plant to complete its life cycle (Seguel, 2014). These mycorrhizal associations increase access to plant nutrients such as: phosphorus (P), nitrogen (N), copper (Cu) and zinc (Zn). The absorption, transport and transfer of P from the mycelium to the plant is fast and efficient due to the presence of carriers with high affinity to the $H_2PO_4^-$ion, which acts coupled with an H^+ symporter carrier through various H^+ -ATPase. On the other hand, fungal mycelium acts in the release of nutrients from particles and mineral rocks by weathering, and also,they connect to the host plants which are the nutrients required for their growth, allowing the flow of energy-rich compounds required for the mobilization of the nutrient; additionally, they increase the absorbing surface area of the plant system, the mycorrhizal extraradical mycelium provides a direct pathway for the translocation of the carbon derived from photosynthesis to the microsites in soil and a large surface area for the interaction of other microorganisms (Finlay, 2008). In this way, the symbiotic nature of the plants with the arbuscular mycorrhizal fungi have proved to be fundamental for the sustainability of the ecosystems, since they are able to colonize large number of terrestrial plants. This technology represents an alternative to improve the soil biological balance and reduce the use of chemical fertilizers and other agrochemical compounds in the production systems (Jeffries *et al.,* 2003).The avocado root lack of absorbent hairs; however, it has been reported that arbuscular mycorrhizal fungi (AMF) colonize the roots of this fruit tree, and that they favor water absorption and use of soil nutrients by the plant, they also promote growth and they keep the nursery plants healthy (Reyes *et al*., 1997; Bárcenas *et al*., 2007).

Published reports on AMF inoculation are scarce and they have shown a broad range of responses, ranging from zero to clear growth responses (Silveira *et al*., 2002),they also improve nutrition, health, growth, resistance to pathogens and tolerance to adverse conditions in the nursery. In avocado seedlings, the application of Glomus spp. Zac-19 and vermicompost favored stem height and diameter (Reyes *et al.,* 1998); in addition, the application of a 1kg fluid paste of the EcoMic® Biofertilizer and 600ml of water, stimulated the development of avocado rootstocks under nursery conditions, which has been reflected on plants of higher quality, and in turn they are a nutritional alternative for this crop (Rivera-Espinosa *et al*., 2011).Avocadosinoculated with AMFin the nursery,increased their height, diameter, and fresh and dry leaves weight; *Rhizophagus fasciculatum* inoculant (used in sterile soil) showed higher growth; on the contrary, *Pacispora scintillans* and*Acaulospora laevis*(on unsterilized soils), showed a decrease in plant growth (Banuelos *et al.,* 2013). On the other hand, in avocado rootstocksinoculated with AMF in the nursery, *Acaulospora delicata* had better plant height and *Scutellospora pellucida*showed larger stem diameter; while *Rhizophagus intraradices* 28-A and*Scutellospora pellucida*increased twice the stem and root weight in relation to other treatments (Carreón *et al*., 2014).In addition, it has been reported that AMFs influence mineral nutrition and carbohydrate content in 'Carmen' avocado seedlings; in all mycorrhizal types, the inoculated plants had higher contents (mg/plant) than the control ones: *S. heterogama* in N, P, K, Mg, Cu and Zn; with *G. etunicatum* in N, P, K, Ca, Mg, Cu and Zn; with *A. scrobiculata* in P, Cu and Zn; and with *G. clarum* in K, Ca, Cu and Zn. All AMF species increased the amounts of carbohydrates in plants (Silveira *et al.,* 2003).

The State of Michoacan, Mexico, produces millions of avocado plants per year in nursery to meet the demand for new plantations at national and regional level, where their quality is highly appreciated. However, the high amounts of fertilizers and pest control products that pollute the environment have been questioned; which is why there is a need to implement new production technologies that reduce these agricultural products, such as the use of organic fertilizers and the application of arbuscular mycorrhizal fungi that have important functions in plant growth (Rivera-Espinosa *et al.,* 2011).

In avocado plantations and nurseries, high amounts of chemical fertilizers and pesticides are used which affect the physicochemical and microbiological properties of soil, they also alter its flora and fauna and because the leached water drags nitrates, then contaminated aquifers and springs cause health problems among consumers. Therefore, it is necessary to look for alternatives in the agricultural activity, more in line with practices that respect nature, that does not harm the health of the consumers and that allow to obtain

http://dx.doi.org/10.22161/ijeab/2.5.59 *ISSN: 2456-1878*

healthy products; for this reason, the aim of this research was to study the behavior of rootstocks of native avocados in nursery and their response to organic nutrition based on bovine and ovine liquid manures and the application of mycorrhizae, in which the following objective was assessed: to evaluate the effect of organic fertilizers on 12 genotypes of native avocados of Ometepec, Guerrero.

II. MATERIALS AND METHODS

Location of studyarea

The study was conducted in Iguala, Guerrero, Mexico, from August to December 2015. The area is located at 757 m altitude, with following coordinates: 18°20'39"N and 99° 29'53" W (GPS Garmin eTrex 10®). The climate is classified as Awo g (w) (i') (Garcia), the driest among the warm subhumids, with rains in summer (June to October), the average annual rainfall is 977.15 mm and the average annual temperature is 25.7°C (García, 1988).

Methodology

Twelve native avocado genotypes (rootstocks) from Ometepec, Guerrero, Mexico, were studied. They were one month old and grown in nurseries on substrates (85% river mud, 5% peat moss and 10% agrolite) that were fertilized with ground (Crusher mill, CH620model, KOHLER®brand) and disinfected (stainless steel steam cooker at 120°C for 30 minutes)ovine and bovinemanures.Also, the commercial mycorrhiza Glumix Irrigation® Biostimulant, and water as control were used. The preparation of manures was 250 gL^{-1} of water and mycorrhiza of 5 g L^{-1} of water; the mixing and dilution of fertilizers and mycorrhizae was done with an SSP mixer [angle grinder (230 mm) (9"), 127 V-15 A 50/60 Hz 6600 r/min, Makita® brand]. The doses of manures and mycorrhiza were 250 mL/ pot, every 30 days, with additional water irrigations every other day.

Variable recording began 15 days after the first application of treatments, then every 30 days: plant height (cm) from neck to stem apex; diameter of stem (mm)at 10 cm in height with a digital vernier (Digimatic calipbre Model: CD-12'CP, Mitutoyo® brand); number of young leaves (NYL), well-formed andnot 100% grown, and green-yellowish to reddish color; number of mature leaves (NML) with 100% growth and intense green color.In October 2015, the chlorophyll content was determined in sunny, young and mature leaves with a SPAD 502 Plus, Minolta, Model B343, Horiba® Brand. In December 2015, from 7- 10:00 am, the petiole extract was obtained from 4 mature leaves/ replicate/ treatment, from which the petiole was cut into portions that were pressed in a garlic press (Kamp® brand); the extracted sap was deposited in the respective ionometers:NO_3^- (METER, Model B-743), K^+ (METER, Model B-731), Ca^{2+} (METER, Model B-751) and Na^+ (METER, Model B-722).

A randomized complete block design was used, which considered four treatments: T1 (sheep manure), T2 (bovine manure), T3 (mycorrhizae, *Glumix Irrigation® Biostimulant*) and T4 (control,common water tap); each of them with four replicates. A variance analysis, a Tukey mean test ($P\leq 0.01$ and 0.05), LSD ($P\leq 0.05$), and a Pearson correlation between the variableswere carried out with the Statistic Analysis System (SAS), version 9.0.

III. RESULTS AND DISCUSSION

Effect of organic fertilizers on the growth and nutritional content of rootstocks

As for organic fertilizers, significant differences ($P\leq 0.01$) were observed for the following variables: plant height, stem diameter, number of leaves (young and mature) and Ca^{2+}. However, chlorophyll in leaves (young and mature), NO_3^-, K^+ and Na^+ showed no significant differences (Table 1).

Plant height and stem diameter

As for height, sheep manure showed the highest value (76.7 cm) of the rootstocks and it was statistically higher than the bovine manure treatment (73.1 cm), which in turn was better to mycorrhizae (69.9 cm), which surpassed the control (63.1 cm cm) (Figure 1). A similar behavior was observed with the stem diameter, where the sheep manure gave the highest value (7.2 mm), and it exceeded the bovine fertilizer (6.8 mm) and mycorrhiza (6.7 mm), which were statistically better than the control (6.3 mm) (Figure 1).

In this research, the mycorrhizae treatment was surpassed by the ovine and bovine fertilizers, in height and stem diameter of the rootstocks. However, these mycorrhizae values in height of the rootstock, exceeded those reported previously(62.6 and 54.4 cm) with Glomus hoi-like and *Glomus mosseae* (Fundora *et al.*, 2011). Mexican avocado landrace rootstocks(*P. americana* Mill. Var. Drymifolia) inoculated with AMF, showed 32.6- 36 cm height values with the application of *Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum* and *Acaulospora scrobiculata* (Castro *et al.*, 2013); whereas as for diameter, in the present study it was observed slightly higher than that reported by Castro *et al.*(2013), who reported an average value of 8.8 cm in diameter with the application of various types of mycorrhizae.

Number of young and mature leaves

The sheep manure compost (6.5 leaves/ plant) showed higher value in young leaves, but it was not statistically superior to the bovine treatments (6.0 leaves/plant) or mycorrhizae (5.9 leaves/plant); it only exceeded the control

http://dx.doi.org/10.22161/ijeab/2.5.59 *ISSN: 2456-1878*

(5.2 leaves/ plant). In relation to the number of mature leaves, sheep manure (18.4 leaves/ plant) was statistically superior to the bovine manure (17.1 leaves/ plant), mycorrhizae (17.1 leaves/ plant) and the control (16.4 leaves/ plant) (Figure 2).

However, in this study,the mycorrhizal treatment was on average as for the number of leaves/plant in comparison to other investigations with (*Glomus hoi-like*) 16.0 and (*Glomus mosseae*) 15.6 leaves/plant (Fundora *et al*., 2011); for consortium of *Glomus fasciculatum, G. constrictum, G. tortuosum, G. geosporum* and *Acaulospora scrobiculata* (21.9 leaves/plant); for the mixture of *G. Mosseae* and *G. cubense*(20.3 leaves/plant) (Castro *et al.*, 2013).

Chlorophyll content in leaves (young and mature)

As for the chlorophyll content in young and mature leaves, the treatments did not show significant effects ($P \leq 0.01$) (Figure 3). However, it is important to mention that young leaves only had half of the chlorophyll content in comparison to mature leaves; maybe because they did not have 100% of the size and they had a coloration between yellowish- green and reddish- green. Therefore, they did not reach their maximum photosynthetic rate (Salisbury and Ross, 1994)due to the immaturity of the stomata (Faust, 1989).In coffee plants, values similar to those found in the present research (40 SPAD units) were reported in adult leaves (Torres-Netto *et al*., 2005); as well as in papaya (*Carica papaya* L.) (Torres-Netto *et al.,* 2002); coffee (*Coffea canephora* P.) (Torres-Netto *et al*., 2005); cotton (*Gossypium hirsutisms* L.) (Brito *et al.,* 2011); pine nut (*Jatropha curcas* L.) (Gonsiorkiewicz *et al.*, 2013); in rice cv. Bing 9363, at the beginning and at panicle maturation, with 40.4 and 35.5 (SPAD units), respectively (Jinwen *et al.*, 2011). In the Hass and Edrenol avocado varieties on patterns of Allesbeste Nursery, Duiwelkloof, of one year old, the chlorophyll content in mature leaves was slightly higher than those obtained in this work, it ranged from 48-57 SPAD units (Bekker *et al*., 2005).

Nutritional content per extract of petiole in treatments

In avocado plants, the nitrate content was extracted in the petiolate where the mycorrhizal treatment was higher (3778 mg L^{-1}), but it was not statistically different from the other treatments, sheep manure (3200 mg L^{-1}), bovine manure (3099 mg L^{-1}) and control (2144 mg L^{-1}) (Figure 4). The results of this research are not similar to those reported for Hass avocado from "El Rosario", municipality of Nuevo Parangaricutiro, Michoacán, where it was found that the N-NO_3 content in leaves was 24.1, 32.1, 25.3 and 47.1 mg L^{-1}, with the application of a fish derivative, organic compost, microorganisms (*Glomus* sp. and *Azospirillum* sp) and vermicompost, respectively (Tapia *et al.*, 2014).In other plant species,lower nitrate contents have been reported to those found in this research; in poblano chili pepper cv. San Luis in Guanajuato, México, 500 mg L^{-1} (Castellanos-Ramos *et al.*, 2001) and 1050 mg L^{-1} of N-NO_3were reported(Brizuela-Amador *et al.*, 2005), whereas in tomato, 2090 mg L^{-1} (Leyva *et al.*, 2005). This suggests that the photosynthetic rate may be high because the nitrogen content is high (Calderón, 1998) and there is a direct relationship between nitrogen and leaf chlorophyll.

The control treatments (2658 mg L^{-1}), mycorrhizae (2658 mg L^{-1}) and bovine manure (2642 mg L^{-1}) (Figure 4), gave potassium results similar to those reported in avocados with the application of Solupotasse (2329.2 mg L^{-1}), Solupotasse + Foliar Solup (2512.0 mg L^{-1}), Granupotasse (2391.7 mg L^{-1}) and Granupotasse + Foliar Solup (2204.2 mg L^{-1}) (Tapia *et al.*, 2007);but they differ from those reported in avocado Hass from "El Rosario", Nuevo Parangaricutiro municipality, Michoacán, where it was found that the K^+content in the leaves was 30.8, 24.4, 26.7 and 54.6 mg L^{-1}, with the application of fish derivative, organic compost, microorganisms (Glomus sp. and Azospirillum sp) and vermicompost, respectively (Tapia *et al.*, 2014). However, sheep manure (3113 mg L^{-1}) showed values higher than those reported in other studies; the differences observed in potassium can be attributed to the variation throughout the yearof temperature, solar radiation and/or relative humidity, in which high and low potassium contents are found according to the seasons (Aguilera *et al.*, 2005).

The sheep manure treatment gave higher calcium content (1496 mg L^{-1}), which exceeded the control (200 mg L^{-1}), but it was statistically similar to bovine manure (530 mg L^{-1}) and mycorrhizae (495 mg L^{-1}), respectively (Figure 4).

As for sodium, all treatments were statistically similar: mycorrhizae (1783 mg L^{-1}), sheep manure (1475.1 mg L^{-1}), bovine manure (1466.7 mg L^{-1}) and control (1321.7 mg L^{-1}). Castro *et al.* (2000) reported lower values for sodium in Nabal, Duke 7 and UCV 7 avocado varieties: 200 mg L^{-1}, 300 mg L^{-1} and 400 mg L^{-1}, respectively.

Conclusions

- The sheep manure affected positively the behavior of rootstocks in plant height, stem diameter, number of young and mature leaves of native avocados of Ometepec, Guerrero.
- The use of organic nutrition did not show a significant response in the chlorophyll content of young and mature leaves.
- Young leaves only reached 50% of chlorophyll content compared to mature leaves.

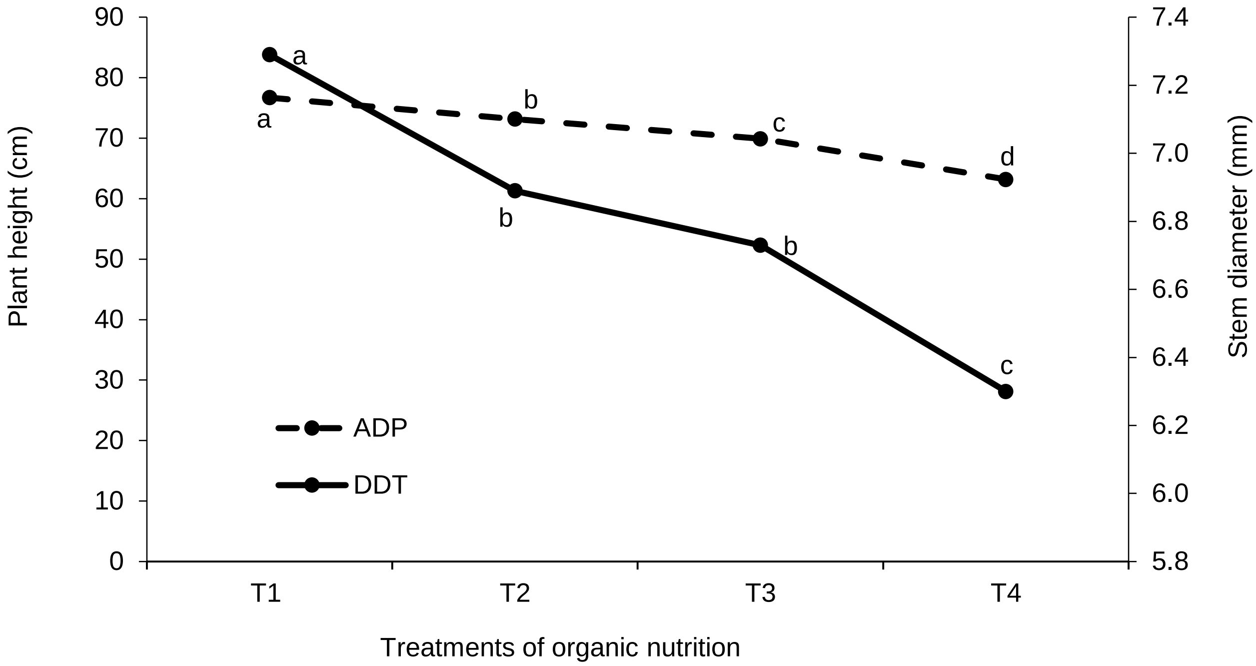

Fig.1: Organic nutrition effect in plant height (PH), stem diameter (SD of genotypes (rootstocks) of native avocados. T1: sheep manure, T2: Bovine manure, T3: mycorrhizae and T4 (control: water) in four replicates, Tukey ($P \leq 0.01$).
hojas/planta) (Castro et al., 2013).

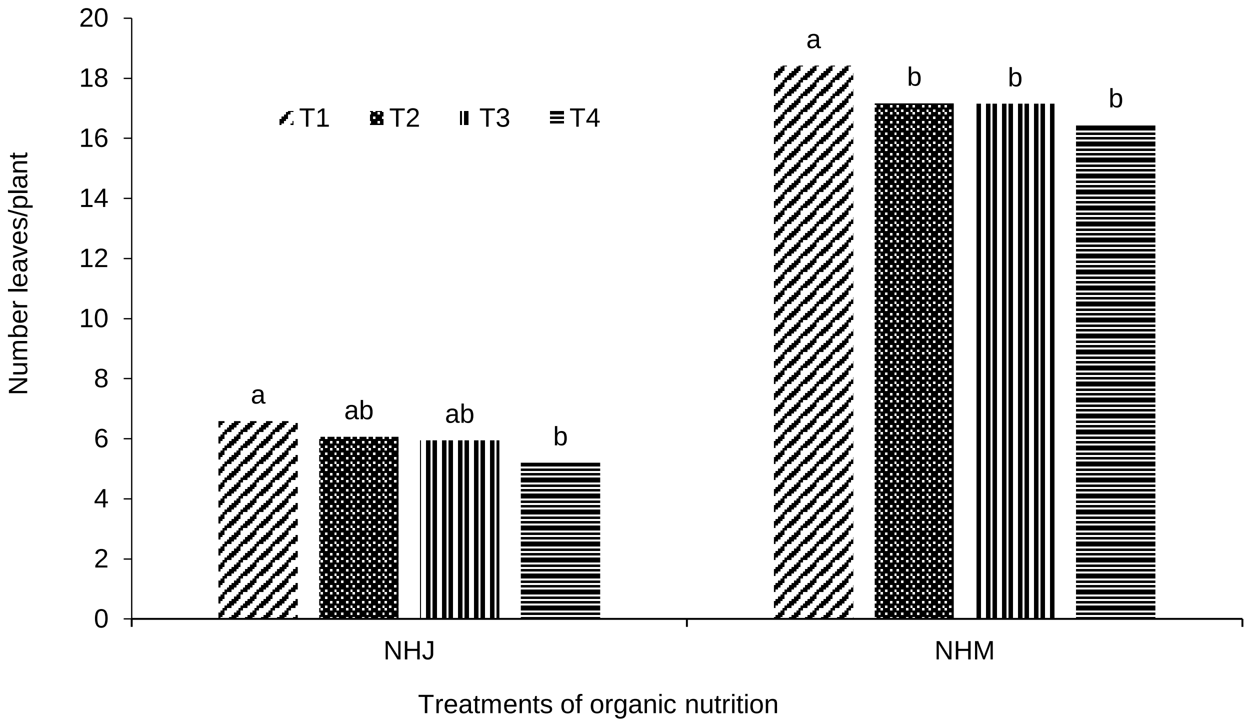

Fig.2: Effect of organic nutrition in number of young leaves (NYL) and number of mature leaves (NML) in native avocados ofOmetepec, Guerrero. T1 (sheep manure), T2 (bovine manure), T3 (mycorrhizae, Glumix Irrigation® Biostimulant) and T4 (control, common water tap), Tukey ($P \leq 0.01$).
SPAD (Bekkeret al., 2005).

http://dx.doi.org/10.22161/ijeab/2.5.59 *ISSN: 2456-1878*

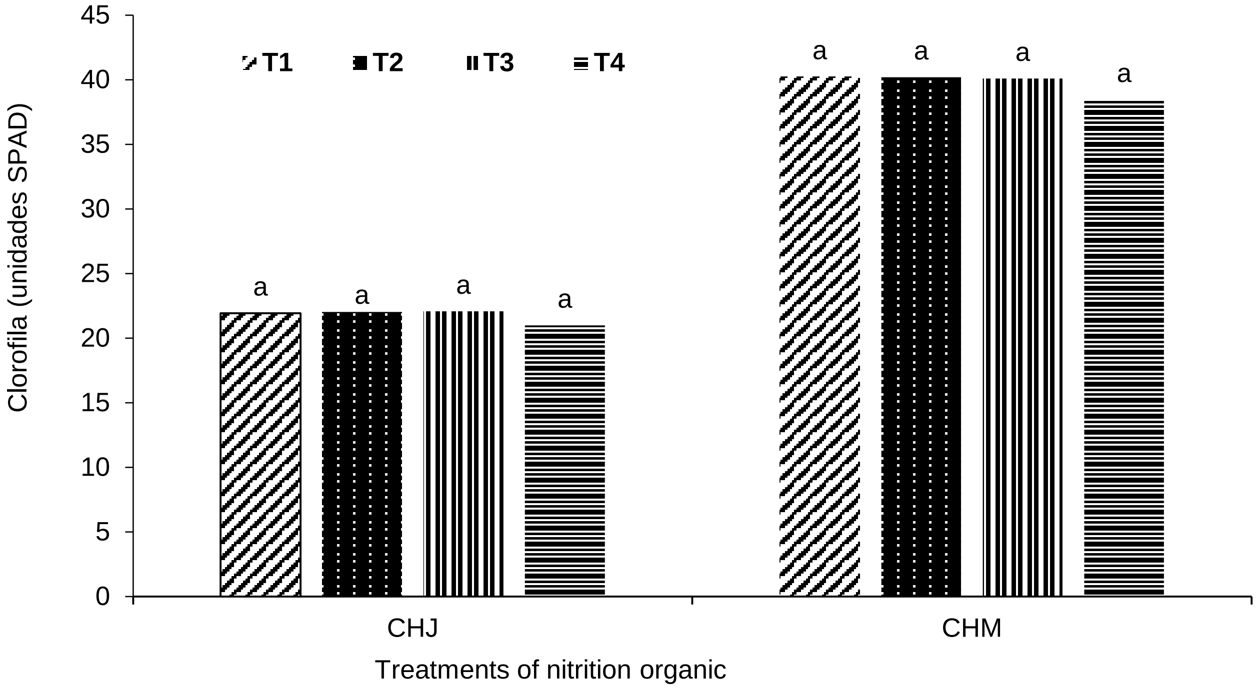

Fig.3: Effect of nitrition organic in content of chlorophyll young leaves (CYL) andchlorophyll of mature leaves (CML) in native avocados of Ometepec, Guerrero. T1 (sheepmanure), T2 (bovinemanure), T3 (mycorrhizae, GlumixIrrigation® Biostimulant) and T4 (control, commonwatertap), Tukey ($P \leq 0.01$).en hojas jóvenes (CHJ) y maduras (CHM) de portainjertos de aguacates nativos del municipio de Ometepec, Guerrero. T1 (estiércol ovino), T2 (estiércol bovino), T3 (micorrizas) y T4 (testigo), Tukey ($P \leq 0.01$).

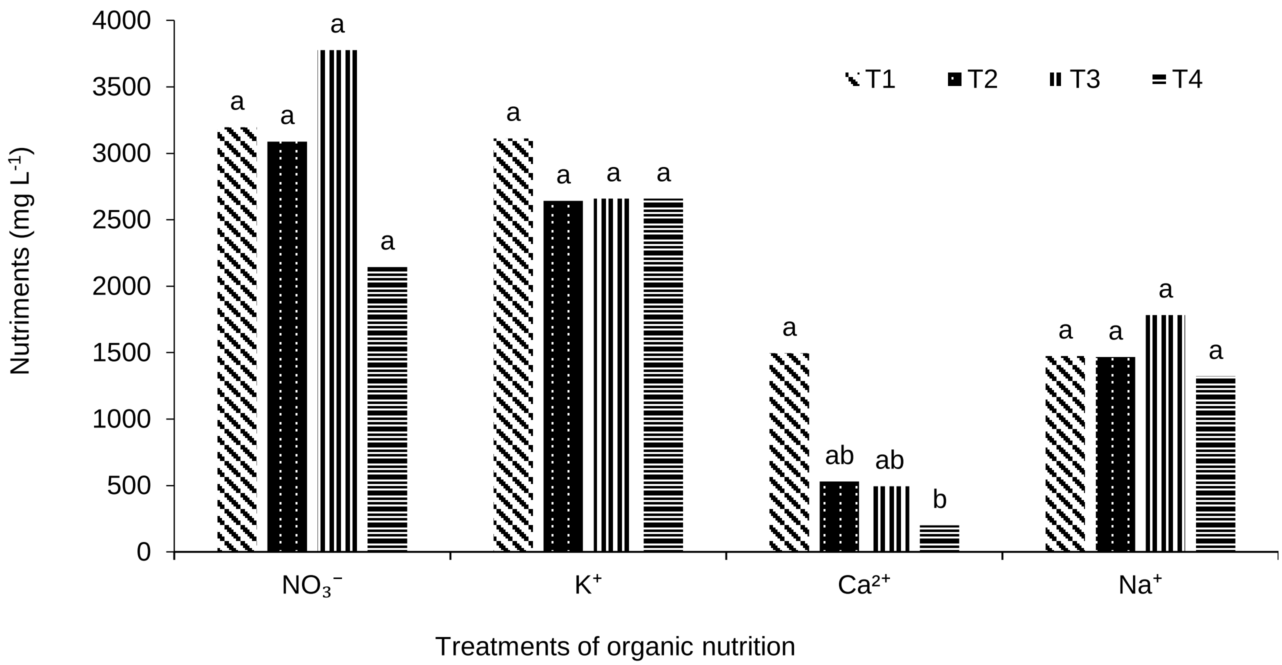

Fig.4: Effect of nutritionorganic in content of ions nitrate, potassium, calcium y sodium in native avocados of Ometepec, Guerrero. T1 (sheep manure), T2 (bovine manure), T3 (mycorrhizae, Glumix Irrigation® Biostimulant) and T4 (control, common water tap), Tukey ($P \leq 0.01$).

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