Response of spinach and Spodoptera exigua to organic and mineral fertilization

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Abstract

Farmers face serious phytosanitary risks and phytosanitary problems, it is generally assumed that the increase of nitrogen (N) in the plant increases the populations of pathogens. The study was carried out in the greenhouse to know the effects of the spinach (Spinacia oleracea L.) variety of Python F1, as well as the response that exists in the oviposition of females and the damage caused by larvae of Spodoptera exigua. It was fertilized with mineral fertilizer based on Steiner nutrient solution (100%, 50%) and with biofertilizers of rabbit leachate (3%) and cachaza compost (1:1 v/v). By means of the ImageJ® program the damaged area of the leaves were calculated and for the leaf area dynamics the PROC NLIN using logistic models. A statistical analysis was performed for agronomic variables, oviposition and spinach damage using Tukey test (p ≤ 0.05). The results showed differences in the fresh weight of the plant (PSP) and the root (PFR), the largest leaf area was the combination treatment of cachaza compost and complete Steiner solution SN (100%), no differences were observed in the treatments at 45 and 60 days after the emergency. Regarding Spodoptera exigua, differences were found in the number of egg masses at 24 h (NMHP24), weight of the larvae (PL), number of damaged leaves (NHD) and area of the damaged leaf (AHD). The rabbit leachate can be an alternative as a biofertilizer for an organic production in spinach.

Keywords: Spinacia oleracea L., biofertilizers, compost, soldier worm.

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Introduction

Mineral or chemical fertilizers are the most used inputs in agriculture, such as the use of urea and phosphate diamonium, these provide nutrients that the plant takes advantage of immediately (Zhang et al., 2016) or organic with the use of biofertilizers such as compost, earthworm humus, animal waste, mineral broths, rock meal, fermented organic fertilizers (Restrepo, 2006; Figueroa-Barrera et al., 2012). The fertilization of the crops, especially of N, tends to increase the biomass and the foliar area. Winter wheat has the best suitable leaf area index through the rational application of fertilizers (Li et al., 2008). The production of seedling biomass of Liquidambar styraciflua L. is significantly related to the specific leaf area, the changes in leaf area and the concentration of leaf N explain 90% of the growth responses (Chang, 2003). The application of N increased the growth and foliar area, the combination of N and phosphorus (P) increased the nutrimental absorption, which only with the contribution of P (Chang, 2003). The leaf area index increased with the density of planting and nitrogen fertilization, with a consequent increase in the yield of the plants (Olsan and Weiner, 2007).

Despite the importance of nitrogen fertilization that is essential in the development and growth of plants (Cease et al., 2012), high doses can bring problems in crops; by modifying the nutritive value of the plants, making them susceptible to attack by phytophagous insects and increasing their populations and the populations of their predators or parasitoids (Yardim and Edwards, 2003; Lu et al., 2007; Aqueel et al., 2015). Some researchers have supported the hypothesis that high doses of nitrogen can result in large levels of damage by herbivores (Altieri, 2007) so that there have been several studies of nitrogen in plants, some mention that this has a relationship with the incidence of pests.

Plants have direct and indirect defenses produced constitutively that induce against pathogens, which can greatly help in their ability to defend themselves. The understanding of plant defense responses to a variety of abiotic stress factors is important to understand the chemical ecology of many parasitoid insects (Ode, 2013). The secondary metabolites of plants play an important role in mediating interactions with insects and their natural enemies; in nature, plants and insects often participate in mutualistic interactions with microorganisms that can also affect secondary metabolism of the plant (Gols, 2014).

The effects of plant-soil feedback on yield may be influenced by nutrient availability; the chemical fertilization and the amount of insects in the plant are interdependent, with changes in the phloem metabolites (Kos et al., 2015b), being different among plant species (Kos et al., 2015b). At higher nitrate doses, more aphids were observed in plants, although the primary metabolites differ slightly (Kutyniok and Müller, 2013).

Volatile plant oils induced by insects are specifically emitted by plants when attacked, these compounds can be perceived by parasitoids and predators that parasitize or feed on insects, among others, including hymenoptera parasites (Becker et al., 2015). The availability of nitrogen (N) can exert a variety of bottom-up effect on plant defense patterns of influencing insect population dynamics, and thus may represent a source of variation in plant-insect interactions (Olson et al., 2009).
Grasshopper nymphs of rice (*Oxya japonica*) that fed on nitrogen-rich and carbon-poor plants, grown on conventional soil, grew and developed more rapidly than those that feed on organically grown plants (Trisnawati *et al*., 2015). The amino acid composition of the phloem exudates was significantly influenced by the chemical fertilization, these changes in the primary and secondary metabolites can be decisive for the insect responses in the plant (Kutyniok *et al*., 2014; Kevi *et al*., 2015).

The nutrients that the plants need allow them to perform biochemical functions, photosynthetic activities that influence the biomass or the reproduction of crops; in the case of spinach, nitrogen is essential in quality, such as color (chlorophyll), as well as vigor and strength in handling during harvest (Aqueel *et al*., 2015; Xing *et al*., 2015; Muchecheti *et al*., 2016).

Luna (1988) mentions that high fertilization increases the incidence of armyworm (*Spodoptera frugiperda* Smith), in *Ostrinia nubilalis* Hübner the results in oviposition were significantly high in the preference of laying eggs on corn in conventional soil with nitrogen fertilizers, in comparison with plants fertilized with manure (Phelan *et al*., 1995). In tomato with high fertilization rate of N, *Frankiniella occidentalis* populations were significantly high (Brodbeck *et al*., 2001). The population of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) increased when it was fed on tomato plants treated with the highest concentration of nitrogen (1.5 mm NO₃⁻) (Larbat *et al*., 2015) Chen *et al*., 2008) showed that the development of *S. exigua* larvae that were fed with cotton plants with application of high doses of nitrogen fertilization was increased and that cotton plants with N levels were chosen preferably by the females of *S. exigua* for the oviposition.

The abuse or scarcity of N in plants can benefit or affect the growth and development of insects; an excess of fertilization leads to an increase in the birth rate, fecundity, longevity, growth and survival of certain pests (Jahn *et al*., 2005; Wang *et al*., 2006). However, when the content of N is low in the plant, the insects that feed on it may be affected, its growth is decreasing, the feeding preference and the survival rate of the pest, e.g. the development of larvae of *S. exigua* fed with cotton plants with a reduction of applications of nitrogen (42 and 112 ppm) was prolonged in relation to the treatments that received higher nitrogen fertilization (196 and 280 ppm) (Chen *et al*., 2008).

The present work had the purpose of observing the response in the spinach growth and phenology, specifically in the leaf area and to observe if the concentration of N in the leaves influences the oviposition and the consumption of larvae L2 of soldier worm (*Spodoptera exiguus* Hübner) manifested as damage to the plant, with mineral fertilization treatments based on Steiner nutrient solution and biofertilizers compost of cachaça and rabbit leachate, under greenhouse conditions.

**Materials and methods**

The work was carried out in a greenhouse at the Postgraduate School, *Campus* Puebla, from March to June 2015. Python F1 variety spinach was used, which is recommended for the Spring-Summer periods. Planting was carried out on March 16, 2015, placing three seeds at a depth of 2.5 cm
in 1 L unicel cups that served as a pot, with soil from the municipality of the Reyes of Juarez, Puebla, to later leave a plant. Irrigation was carried out according to the treatments depending on the needs of the plants.

**Treatments**

The trial consisted of six treatments with five repetitions, distributed in a completely randomized design, which consisted of the following: Steiner 100% nutrient solution (SN100%) and 50% (SN50%), 100% Steiner nutrient solution with the addition of cachaça compost (SN+COMP), cachaça compost (COMP) and rabbit leachate (LIXCON) and without fertilization (control). The composition of the Steiner solution for 200 L of water was with the sources potassium nitrate (63 g), calcium nitrate (120 g), monopotassium phosphate (30 g), magnesium sulfate (75 g), potassium sulfate (67 g), H₂SO₄ (20 ml) and micronutrients (10 g) Ultrasol® micro mix (Fe 7.5%, Mn 3.7%, B 0.7%, Zn 0.6%, Cu 0.3% and Mo 0.2%). The cachaza compost used contains 2.56% N, pH of 7.84, CE of 3.5, dS m⁻¹, P 767.2 ppm, Fe 88.6 ppm, Mn 26.6, Zn 20.3 ppm and Cu 8.841, while the leached rabbit contains pH of 9.84 and CE of 11.011 dS m⁻¹, P 37.2 ppm, Fe 0.062 ppm, Mn 0.042, B 0.25 ppm, Zn 0.0.24 ppm, Cu 0.01 and Mo 0.011 ppm.

**Foliar area dynamics**

Adjustments were made to the dynamics of leaf area development (FOL) considering non-linear or logistic models (Hunt, 1982), using non-linear regression techniques, using the procedure PROC NLIN of the statistical program SAS see. 9.0 (SAS Institute, 2002). The estimation of the leaf area was made by means of an adjustment factor, 15 leaves of 5 plants were selected, taking the real area of the leaf from direct measurement where the length (L in cm) and width (A in cm) of the leaves (Hoyos et al., 2005; Cabezas-Gutiérrez, 2009) and the adjustment factor was obtained.

The logistic model used to estimate the leaf area dynamics: \( Y = \frac{A}{1 + Be^{-CX}} \). Where: A, B and C are parameters whose values are obtained with the PROC NLIN program in the SAS; X= the days after sowing and; \( e \)= natural logarithm.

The agronomic variables to be measured were: number of leaves, length and width of the leaves (cm), during the test 4 measurements were made every 20 days after sowing; At the harvest, height of the plant (cm), length of the root (cm), fresh weight of the plant (g) and root (g) were measured, in the case of dry weight of the plant and root were subsequently to harvest. Visual observations were made looking for the morphological characteristics described by the BBCH scale for phenology (Meir, 2001), so it was considered the change of a phase or stage when 50% of plants per treatment presented them. In the case of Spodoptera exigua the variables to be studied were: number of egg masses and number of eggs in spinach in terms of time (24, 48 and 72 h), number of damages per leaf, total area of the leaf and the sum of the damaged area of the leaves.

**Analysis of total nitrogen in spinach**

The determination of the nitrogen content in the leaves was made in dry plant tissue, the samples were crushed with an A11 Basic®, grain mill, using accessories for shock grinding to have homogeneous samples and determined in the Laboratories Unit (Ulabs) of the Postgraduate School-Campus Puebla through the micro Khendalj method (Ortega et al., 2006).
Exposure of females and larvae of *Spodoptera exigua* in spinach

In February 2015 larvae of different instars of *Spodoptera exigua* were collected in spinach culture in the municipality of The Reyes of Juarez, Puebla. The colony was established and fed artificially modified diet of Budia *et al.* (1994) in the Food Safety and Phytosanitary Laboratory of the Postgraduate College-Campus Puebla.

The 24-h-old females and males were selected, placing them in waxed bags 10 females and 1 male, they were left for 48 h for fertilization. In a non-selection trial, 30 fecund females were selected, having female/plant which were placed inside wooden cages (30 x 30 x 30 cm) lined with organza fabric (Ortega-Arenas *et al.*, 2006), placing the females within these at 56 days after the germination of the plants of each treatment. The females were fed with a 10% water and honey solution. The eggs were removed and counted every 24 h (Belda *et al.*, 1994). By means of a stereoscopic microscope (Motic® DM143) the number of eggs per mass was counted and the emergence of the larvae was expected. We selected 30 larvae in L2 at random, placing one larva per plant to avoid competition and cannibalism, were placed 64 days after sowing the spinach until the harvest date. In this stage the damaged leaves per plant in each treatment were reviewed.

**Leaf damage analysis by *Spodoptera exigua***

Spinach leaf damage analysis was performed with the ImageJ® program proposed by Abramoff *et al.* (2004) through the analysis of images that allowed to determine the values of the leaf area as the damaged surface of the leaf by the insect (Rasband, 2009; Ferreira and Rasband, 2011; Sauceda-Acosta, 2015). In the harvest, digital photographs of the leaves with damage were taken, placing the leaf on a white and flat base, as well as a ruler graduated in centimeters at the base of the plant as a reference for the measurements and to calibrate the image in the program, which is necessary for the adjustment in the pixel scale, selecting the unit of cm for the measurement. For the calculation of the total area of the leaf and the damaged area, the area data were passed to Microsoft Office Excel® 2010 and analyzed statistically in SAS (SAS Institute, 2002).

**Results and discussion**

**Agronomic variables**

The results showed statistical differences in the number of leaves, fresh weight of the plant, dry weight of the plant, length of the root and height of the plant, the best treatment was SN+COMP (table 1), in this treatment they were observed with dark green color, followed by LIXCON both showed good flexibility in the leaves, which gives them a better spinach appearance, as a good indicator of quality (Burt, 1997) while the other treatments presented a green color, lemon with brittle leaves. However, LIXCON was the only treatment that reached flowering, observing the growth of the stem in the middle of the rosette, this response may be influenced by the photoperiod and temperature greater than 20 °C (Arana and Marenco, 2003; González *et al.*, 2004), on the other hand there is evidence that phosphorus favors spinach flowering (Serrano, 1976). The early flowering in spinach is a response that does not favor the harvest, since it seeks to obtain the highest vegetative production (Jiménez *et al.*, 2010).
Table 1. Response of different agronomic characters of spinach to organic and mineral fertilization.

<table>
<thead>
<tr>
<th>TRT</th>
<th>NH</th>
<th>PFP (g)</th>
<th>PFR (g)</th>
<th>PSP (g)</th>
<th>PSR (g)</th>
<th>LR (cm)</th>
<th>AP (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN (100%)</td>
<td>28 ±3.162</td>
<td>22.654</td>
<td>4.12 ±3.32</td>
<td>4.386</td>
<td>0.596 ±0.227</td>
<td>21.112</td>
<td>15.464</td>
</tr>
<tr>
<td></td>
<td>ab</td>
<td>±5.956 ab</td>
<td>ns</td>
<td>±1.164 ab</td>
<td>ns</td>
<td>±3.42 a</td>
<td>±1.026 ab</td>
</tr>
<tr>
<td>SN (50%)</td>
<td>28.6</td>
<td>19.604</td>
<td>2.23 ± 0.991</td>
<td>4.436 ±0.41</td>
<td>0.714 ±0.379</td>
<td>22.774</td>
<td>14.852</td>
</tr>
<tr>
<td></td>
<td>±2.302 ab</td>
<td>±3.794 ab</td>
<td>a</td>
<td>ab</td>
<td>a</td>
<td>±4.28 a</td>
<td>±1.021 b</td>
</tr>
<tr>
<td>SN+COMP</td>
<td>30 ±5.708</td>
<td>27.962</td>
<td>6.404</td>
<td>4.836</td>
<td>0.756 ±0.235</td>
<td>23.524</td>
<td>17.86 ±1.276</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>±2.486 a</td>
<td>±2.638 a</td>
<td>±0.632 a</td>
<td>a</td>
<td>±4.782 a</td>
<td>a</td>
</tr>
<tr>
<td>COMP</td>
<td>21.6</td>
<td>15.332</td>
<td>1.422</td>
<td>3.302</td>
<td>0.392 ±0.133</td>
<td>9.556</td>
<td>14.576</td>
</tr>
<tr>
<td></td>
<td>±1.516 bc</td>
<td>±3.915 b</td>
<td>±0.799 a</td>
<td>±0.876 b</td>
<td>a</td>
<td>±4.142 b</td>
<td>±1.734 b</td>
</tr>
<tr>
<td>LIXCON</td>
<td>21.4</td>
<td>23.98</td>
<td>4.6 ±1.133</td>
<td>3.97 ±0.582</td>
<td>0.708 ±0.074</td>
<td>21.674</td>
<td>16.32 ±1.809</td>
</tr>
<tr>
<td></td>
<td>±5.595 bc</td>
<td>±10.125 ab</td>
<td>±3.915 b</td>
<td>±0.799 a</td>
<td>±0.876 b</td>
<td>±4.142 b</td>
<td>±1.734 b</td>
</tr>
<tr>
<td>Control</td>
<td>17.2</td>
<td>15.966</td>
<td>3.388</td>
<td>4.07 ±0.612</td>
<td>0.748 ±0.171</td>
<td>20.874</td>
<td>15.56 ±1.7</td>
</tr>
<tr>
<td></td>
<td>±4.147 c</td>
<td>±3.493 b</td>
<td>±1.744 a</td>
<td>±0.672 a</td>
<td>±2.599 a</td>
<td>±2.861 a</td>
<td>ab</td>
</tr>
<tr>
<td>DHS</td>
<td>7.9273</td>
<td>11.062</td>
<td>5.2495</td>
<td>1.4725</td>
<td>0.4377</td>
<td>7.3598</td>
<td>2.8669</td>
</tr>
</tbody>
</table>

TRT= treatment; SN (100%)= 100% Steiner solution, SN (50%)= 50% Steiner solution; SN+COMP= Steiner solution plus cachaza compost; COMP= cachaza compost; LCO= rabbit leachate; NH= number of leaves, PFP= fresh weight of the plant; PFR= fresh weight of the root; PS = dry weight of the plant; PSR= root dry weight; AP= height of the plant and LR= length of the root, DHS= significant honest difference. Treatments with the same letter in the column do not differ significantly Tukey ($p \leq 0.05$)

Leaf area dynamics

The leaf area (FOL) of the spinach was adjusted to a logistic model in all the treatments (Table 2 and Figure 1), allowing to appreciate the growth that the spinach developed through the treatments. The differences are presented at 45 and 60 days. The treatment with greater leaf area was SN+COMP at 52 days after sowing, the combination of cachaza compost and 100% Steiner solution favors the spinach leaf development, Van Cleempul and Hera (1996) cited by Aguilar (2005) mention that chemical fertilization has a limited efficiency, because the crops only absorb between 10 and 50% of the applied fertilizer, while the organic fertilizer improves soil conditions so the combination of both could help in the growth and development of spinach. However, LIXCON showed better differences at 30 days after emergence showing greater leaf area than COMP, works in Colombia report that the use of rabbit provides better yields in kg m$^{-2}$ in spinach compared to other composts of animal organic waste. since the contribution of nutrients is the most suitable for this crop (Jiménez et al., 2010; Figueroa-Barrera et al., 2012).

The smallest growth was shown by the control in which yellow leaves appeared 45 days after the emergence, to this Jimenez et al. (2010) mentions that spinach develops few leaves with a smaller size than normal that eventually turn yellow and is due to low concentrations of N in the development medium.
Table 2. Growth models of the foliar area (FOL) with respect to mineral and organic fertilization in spinach.

<table>
<thead>
<tr>
<th>TRT</th>
<th>Foliar area (mm$^2$) estimated model</th>
<th>Fcal</th>
<th>Prob</th>
<th>Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (14 dds)</td>
</tr>
<tr>
<td>SN (100%)</td>
<td>FOL=37846/1+145.2e$^{-0.1029x}$</td>
<td>264.11</td>
<td>&lt;0.0001</td>
<td>137.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±53.1</td>
<td>±2100.4</td>
<td>±854.4</td>
</tr>
<tr>
<td>SN (50%)</td>
<td>FOL=42071.7/1+118e$^{-0.9907x}$</td>
<td>539.09</td>
<td>&lt;0.0001</td>
<td>93.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±37.3</td>
<td>±1620.6</td>
<td>±4219.2</td>
</tr>
<tr>
<td>SN+COMP</td>
<td>FOL=50426.9/1+138.3e$^{-0.0878x}$</td>
<td>887.21</td>
<td>&lt;0.0001</td>
<td>154.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±30.5</td>
<td>±1396.6</td>
<td>±3626.3</td>
</tr>
<tr>
<td>COMP</td>
<td>FOL=30752.8/1+121.2e$^{-0.0895x}$</td>
<td>88.47</td>
<td>&lt;0.0001</td>
<td>110.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±21.7</td>
<td>±2264.2</td>
<td>±8079.7</td>
</tr>
<tr>
<td>LIXCON</td>
<td>FOL=34676.2/1+79.83e$^{-0.0875x}$</td>
<td>122.07</td>
<td>&lt;0.0001</td>
<td>121.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±31.5</td>
<td>±1803.9</td>
<td>±8083.8</td>
</tr>
<tr>
<td>Control</td>
<td>FOL=29076/1+81.335e$^{-0.0837x}$</td>
<td>123.59</td>
<td>&lt;0.0001</td>
<td>173.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±79.8</td>
<td>±1061.4</td>
<td>±6557.7</td>
</tr>
<tr>
<td>DHS</td>
<td></td>
<td>96.7</td>
<td>2004.6</td>
<td>3178.3</td>
</tr>
</tbody>
</table>

TRT= treatment; SN (100%)= 100% Steiner solution; SN (50%)= 50% Steiner solution; SN+COMP= Steiner solution plus cachaza compost; COMP= cachaza compost; LIXCON= rabbit leachate, FOL= leaf area; X= days after sowing (dds); e= natural logarithm (2.718281828). †DHS= significant honest difference.

Figure 1. Spinach leaf area dynamics in different fertilization treatments, cultivated in soil. The applied treatments= SN (100%)= 100% Steiner solution; SN (50%)= 50% Steiner solution; SN100%+COMP= Steiner solution + cachaza compost; COMP= cachaza compost; LIXCON= rabbit leachate and control.

Exposure of females and damage in spinach leaves by L2 larvae of Spodoptera exigua

During the test with S. exigua females, significant differences were found in the number of egg masses at 72 h (NMH72) and in the number of eggs per plant at 72 h (NHP72) found in spinach leaves (Table 3). Similar results were obtained by Ortega-Arenas et al. (2006) in non-choice tests,
where they indicate that oviposition was not affected by the dose of N, but there are factors that can influence the rate of oviposition such as the water content and N in the leaves, characteristics of the plant, age of leaves and population density; Kevi et al. (2015), found that aphid populations in young walnut plants do not depend on N, but on other nutrients or other abiotic factors. Stafford et al. (2012) report that the population of insects in plants that grow in manure is lower, compared to those that grow with ammonium nitrate.

Table 3. Oviposition response of *Spodoptera exigua* in different treatments with organic and mineral fertilization in spinach under greenhouse conditions (number of masses and number of eggs in different periods of time).

<table>
<thead>
<tr>
<th>TRT</th>
<th>Num. of egg masses per plant (NMHP)</th>
<th>Num. of eggs per plant (NHP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 (h)</td>
<td>48 (h)</td>
</tr>
<tr>
<td>SN (100%)</td>
<td>0.8 ±0.447 ns</td>
<td>1.6 ±0.894 ns</td>
</tr>
<tr>
<td>SN (50%)</td>
<td>0.6 ±0.548 a</td>
<td>1.6 ±1.14 a</td>
</tr>
<tr>
<td>SN+COMP</td>
<td>1 ±0.707 a</td>
<td>1.4 ±0.548 a</td>
</tr>
<tr>
<td>COMP</td>
<td>0.6 ±0.548 a</td>
<td>0.8 ±0.837 a</td>
</tr>
<tr>
<td>LIXCON</td>
<td>0.6 ±0.548 a</td>
<td>0.8 ±0.447 a</td>
</tr>
<tr>
<td>Control</td>
<td>1.2 ±1.095 a</td>
<td>0.6 ±0.548 a</td>
</tr>
<tr>
<td>DHS</td>
<td>1.3359</td>
<td>1.5147</td>
</tr>
</tbody>
</table>

TRT= treatment; SN (100%)= 100% Steiner solution; SN (50%)= 50% Steiner solution; SN+COMP= Steiner solution plus cachaza compost; COMP= cachaza compost; LIXCON= rabbit leachate; DHS= significant honest difference. Treatments with the same letter in the column do not significantly differ Tukey (p≤ 0.05).

Regarding the damage that the second instar larvae can cause in spinach, they are very noticeable, showing a preference for the leaves that are in the middle of the spinach rosette; significant differences were found in the weight of larvae (PL), number of damages (ND) and total area of the leaves with presence of damage (ATH) and in damaged area of the leaf (ADH); with respect to the percentage of total N, all the spinach plants with treatment had a greater amount of N than the control plants, and in the same way they presented a greater number of leaves (NH). The larvae fed in the LIXCON, SN100% and COMP treatments had the highest weights and next to pupate.

Regarding the weight of the larvae, no relationship was found with the total% N in the leaf (Table 4). However, there may be secondary metabolites that provide essential nutrients for *S. exigua* (Saeed et al., 2009). Gash (2012), in winter wheat with recommended levels of fertilization found that there was a growth rate and aphid fecundity, with the increase in fertilization; however, at higher doses the decrease is significant in fertility due to the greater application of fertilizers (Figueroa-Brito et al., 2013; Flores-Macías et al., 2016).
Table 4. Damage to spinach leaves by 2nd instar larvae. Effect of Steiner solution application, cachaca compost and rabbit leachate in the posture of egg masses in plant, total egg masses in the whole system and the number of eggs in plant.

<table>
<thead>
<tr>
<th>TRT</th>
<th>PL (g)</th>
<th>(%)N</th>
<th>NHD</th>
<th>ND</th>
<th>ATH (cm²)</th>
<th>ADH (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN (100%)</td>
<td>0.148 ±0.058</td>
<td>a</td>
<td>3.161 ±0.098</td>
<td>17.4 ±2.302</td>
<td>98.76 ±49.864</td>
<td>255.82 ±73.894</td>
</tr>
<tr>
<td>SN (50%)</td>
<td>0.124 ±0.053</td>
<td>ab</td>
<td>2.931 ±0.08 a</td>
<td>16.4 ±5.459</td>
<td>60.2 ±17.152 b</td>
<td>234.78 ±76.509 ab</td>
</tr>
<tr>
<td>SN+COMP</td>
<td>0.086 ±0.049</td>
<td>b</td>
<td>2.512 ±0.08 b</td>
<td>16.8 ±9.97 a</td>
<td>123.6 ±38.507 a</td>
<td>306.69 ±63.162 a</td>
</tr>
<tr>
<td>COMP</td>
<td>0.138 ±0.062</td>
<td>ab</td>
<td>2.522 ±0.262</td>
<td>17.8 ±2.193 a</td>
<td>85.2 ±12.438 ab</td>
<td>167.36 ±71.041 b</td>
</tr>
<tr>
<td>LIXCON</td>
<td>0.204 ±0.056</td>
<td>a</td>
<td>2.973 ±0.128</td>
<td>17.4 ±5.319 a</td>
<td>91.2 ±30.128 ab</td>
<td>208.41 ±65.061 b</td>
</tr>
<tr>
<td>Control</td>
<td>0.096 ±0.032</td>
<td>b</td>
<td>1.884 ±0.077</td>
<td>11.2 ±3.271 a</td>
<td>49.04 ±17.404 b</td>
<td>134.56 ±51.414 b</td>
</tr>
<tr>
<td>DHS</td>
<td>0.103</td>
<td>c</td>
<td>0.269</td>
<td>8.3348</td>
<td>59.895</td>
<td>131.73</td>
</tr>
</tbody>
</table>

TRT= treatment; SN (100%)= 100% Steiner solution; SN (50%)= 50% Steiner solution; SN+COMP= Steiner solution plus cachaza compost; COMP= cachaza compost; LCO= rabbit leachate; NH= number of leaves; % N= percentage of nitrogen; NHD= number of damaged leaves; ND= number of damages; ATH= total area of the leaves with presence of damage; ADH= damaged area of the sheet, DHS= significant honest difference. Treatments with the same letter in the column do not significantly differ Tukey (p≤0.05).

The damages of \textit{S. exigua} were very noticeable, showing a preference for the leaves that are in the middle of the rosette. SN+COMP and LIXCON had greater leaf area as well as the largest damaged area, at the time of harvest. Spinach plants sprinkled with SN50% or LIXCON registered a similar percentage of N, but the behavior of \textit{S. exigua} larvae on spinach leaf damage was different. This effect may be due to the presence or absence of the other nutrients of each solution. According to Stafford \textit{et al.} (2012), the effect of the type of fertilizer applied to the plant can affect the insect population; likewise, the applied dose may increase the parasitism population of \textit{S. exigua} (Chen \textit{et al.}, 2014).

Conclusions

In the evaluated agronomic variables, the combination of nutritious solution and cachaza compost stimulate the growth and weight of the spinach plant, likewise it was observed that the leaf length and greener color was higher in comparison with the rest of the treatments. Of the organic fertilizers used, rabbit leachate can be an alternative as a biofertilizer for an organic production in the spinach crop. No relationship was found between the oviposition of \textit{S. exigua} and the total N concentration in the foliage of the spinach. The combination of the complete nutritious solution with organic fertilizer presented greater damage of \textit{S. exigua} for having greater leaf area.

Acknowledgments

To spinach producers in the municipality of The Reyes of Juarez, Puebla.
Cited literature


Rasband, W. S. 2009. ImageJ, Mac OS X. National Institute of Mental Health, Bethesda, Maryland, USA.


