

Botanical formulations for the ecological management of *Myzus persicae* (Sulzer) and *Aphis gossypii* (Clover) (Hemiptera: Aphididae) and their side effects on parasitoids

Formulados botánicos para el manejo sustentable de *Myzus persicae* (Sulzer) y *Aphis gossypii* (Clover) (Hemiptera: Aphididae) y sus efectos secundarios en parasitoides

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ARTICLE DATA

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ABSTRACT

The peach aphid *Myzus persicae* (Sulzer) and cotton aphid *Aphis gossypii* (Clover) (Hemiptera: Aphididae) are considered to be key pests affecting greenhouse pepper crops in Argentina as a result of their frequent occurrence and the seriousness of the damage caused by their feeding behavior and the transmission of virus. The goal of this research was to determine the efficiency of botanical products to control aphids and their side effects on parasitoids in this crop. Thus, three biorational pest control formulations derived from essential oils (EO) and plant extracts (Es) were tested, namely (i) neem EO, cinnamon EO, clove EO, oregano EO and American marigold EO (formulation 1); (ii) garlic EO and cinnamon EO (formulation 2); (iii) garlic E and rue E (formulation 3); and a soy lecithin adjuvant (lecithin), and finally, a control (water spray method). For this research, a completely randomized design was replicated 3 times. These treatments were applied directly to the foliage by means of a backpack sprayer on a weekly basis until the end of this trial. Subsequently, the total number of healthy aphids and parasitized aphids (mummies) on every leaf was recorder in the field and in the laboratory analyzed thorough repeated measures. Analysis of Variance (ANOVA) and LSD Fisher method. The results showed that formulation 1 and formulation 3 recorded a lower number of aphids and mummies compared to the other treatments. This evidence would demonstrate that these formulations repel aphids and parasitoids without the lethal effects caused by the use of broad spectrum insecticides.

Key words: aphids; extracts; essential oils; parasitoids.

RESUMEN

Los pulgones del duraznero *Myzus persicae* y del algodónero *Aphis gossypii* (Hemiptera; Aphididae) son plagas claves en el cultivo de pimiento protegido en Argentina debido a su frecuente aparición y la magnitud de los daños ocasionados por la alimentación y la transmisión de virus. El objetivo del trabajo fue determinar la eficacia de productos botánicos

para controlar esta plaga y sus efectos secundarios en parasitoides en este cultivo. Para ello, fueron evaluados tres formulados de origen botánico para control de plagas derivados de aceites esenciales (AEs) y extractos vegetales (Es), a saber: (i) aceite esencial (AE) de neem, AE canela, AE clavo de olor y combinaciones: AE orégano y AE de copete (formulado 1); (ii) AE de ajo y AE de canela (formulado 2); (iii) extracto (E) de ajo y E de ruda (formulado 3); una coadyuvante lecitina de soja (lecitina) y, una pulverización con agua (control). Para este estudio se efectuó un diseño totalmente aleatorizado con tres repeticiones. Los tratamientos fueron aplicados directamente sobre el follaje con mochila manual semanalmente hasta concluir el estudio. Posteriormente, se evaluó el número de pulgones sanos y parasitados (momias) por hoja en campo y laboratorio mediante un Análisis de Varianza (ANOVA) de medidas repetidas y test LSD Fischer. Estos resultados mostraron que los formulados 1 y 3 registraron un menor número de pulgones y momias comparados con los restantes tratamientos. Esta evidencia indicaría que estos formulados repelen pulgones y parasitoides, sin generar la letalidad que producen los insecticidas de amplio espectro.

Palabras clave: pulgones; extractos; aceites esenciales; parasitoides.

INTRODUCTION

In the Province of Entre Ríos, Argentina, the peach aphid *Myzus persicae* and the cotton aphid *Aphis gossypii* have easily settled in greenhouse pepper production in response to optimal environmental conditions for their development. The rapid increase of these populations gives rise to two types of damage: (1) direct damage as a result of sap feeding by adults and nymphs (Jarošvá *et al.*, 2016) and (2) indirect damage due to the development of sooty mold - commonly known as "fumagina", which grows as a consequence of the carbohydrates excreted by nymphs and adults (SENASICA, 2014).

Although this substance causes considerable indirect damage because it weakens the plant and decreases its photosynthetic rates (Santos *et al.*, 2013), the damage caused by the transmission of diseases by aphids, as vectors of diverse viruses in different crops, is even worse (Blackman and Eastop, 2017). Since only few studies related to significant pest management tools enhancing the conservation of natural enemies have been found, it was decided to study one specific tool in greenhouse pepper crops in the city of Concordia, Provincia de Entre

Ríos. Thus, a survey of a variety of natural enemies prior to the first stage of this research has been required. The species identified were *Aphidius colemani* (Viereck), *Diaeretella rapae* (Mcintosh), *Praon Volucre* (Holiday), *Aphelinus abdominales* (Dalman) (Hymenoptera Aphelinidae) and *Praon volucre* (Hymenoptera: Braconidae), as parasitoids of *M. persicae* and *A. gossypii* in the city of Concordia R. Paz, (personal communication, april 4, 2017) Currently, the use of synthetic pesticides is the usual method to control this pest; however, this strategy is not only inefficient (Bass *et al.*, 2014) in horticultural crops, but also harmful for both the environment and human life (Costa, 2018). As a result, botanical insecticides deriving from EOs and Es - with various active compounds (secondary metabolites) - may offer a suitable alternative to chemical pesticides (Villaverde *et al.*, 2014).

EOs are complex mixtures of diverse natural substances, for instance, monoterpenes, sesquiterpenes, phenols, oxides, ethers, alcohols, esters, aldehydes and ketones. However, most of these mixtures contain two or three main chemical components classified under two chemical groups such as terpenoids (monoterpenes and sesquiterpenes) and

phenylpropanoid compounds (Asbahani *et al.*, 2015), which - according to their combinations - may be biologically active to act synergistically as bactericides, fungicides, and insecticides (Tak and Isman, 2017). These components, which are part of EOs, enhance the crop protection since they act as a deterrent against feeding and adult oviposition that leads to an acute toxicity and the interruption of the developmental stages of these insects (Molina, 2001).

Therefore, these components provide an interesting alternative control that decomposes easily in the environment and is safer compared to synthetic pesticides (Pavela and Benelli, 2016). This research aimed to determine the efficiency of three botanical formulations for the management of *A. gossypii* and *M. persicae* and their effect on the parasitoid population in greenhouse pepper crops.

MATERIALS AND METHODS

This research was carried out in a greenhouse located in a horticultural section of the Argentine Agricultural Experiment Station (EEA) of the National Institute for Agricultural Technology (INTA) of the city of Concordia, Province of Entre Rios (Argentina), located at 31°22'22.16"S; 58°7'5.42"W; 396 (metres above sea level) GPS coordinates. In March 28, 2016, the sweet pepper (*Capsicum annum* L.), ZEN F1 commercial hybrid (Clause) was transplanted in biosolarized soils within a plantation area of 1.20 metres between rows and 0.40 metres between plants. The plants were placed in a trellis structure and after one week, they were nourished with Nutrire Plus® fertilizer at a rate of 3 litres/100m²/ on a weekly basis.

Three formulations derived from EOs and Es, an adjuvant and one control (Table 1).

Table 1. Treatments assessed for pest management.

Product	Composition (%)	Dosage	Application
Formulation 1	AEs (<i>Azadirachta indica</i> A. juss.) 32%, (<i>Cinnamomum verum</i> J.Presl) 24%, (<i>Tagetes erecta</i> L.) 8%, (<i>Origanum vulgare</i> L.) 4%, and (<i>Syzygium aromaticum</i> L.) 2%.	5 ml/L water	On the aerial part of the plant
Formulation 2	AE (<i>Allium sativum</i> L.) 30% and EP (<i>C. verum</i> J.Presl) 30%	5 ml/L water	
Formulation 3	AE (<i>A. indica</i> L.) 70%, EPs (<i>A. sativum</i> L.) 25 % and (<i>Ruta graveolans</i> L.) 5%.	5 ml/L water	
Lecithin	Soybean lecithin-based adjuvant (Lecithin Max®)	2 ml/L water	
Control	Water spray		

These treatments were sprayed directly into the foliage at sunset - low intensity of sunlight - and during the crop cycle with an interval time of 7-10 days from April to November, 2016, using a 20 litre symmetrical backpack sprayer (Guarany® brand) equipped with a cone nozzle. Botanical products easily decompose when exposed to high temperatures, ultraviolet light and evaporation. Therefore, during the application -including the control treatment- a dose of 0.2% of soybean lecithin adjuvant was added (Lecithin Max®) to enhance the adhesion, penetration, and anti-evaporation. This product was donated by the company named Fragaría S.R.L (Villa Cañas, Santa Fe, Argentina). Prior to the application of these treatments, a monitoring process was carried out on a weekly basis as from the 16th day after the transplanting (DAT).

These monitoring inspections determined whether the aphids (*Aphis gossypii* (Glover), *Myzus persicae* (Sulzer) were found above the damage threshold (DT) or not with an average number higher than 8 (nymphs + adults) per leaf (Polack and Mitidieri, 2005). Then it was decided to apply a treatment with a biorational pesticide based on [NeemAzal-T/S® (0.4% azadirachtin)] combined with [Hydralene® (0.2% potassium oleate)] to avoid causing serious damage to the crop.

In order to carry out this research, five raised beds of 0.50m wide and 28m long were built. Each of them comprised 3 plots of land, where 15 plants were placed. The plots of land were separated by transition zones or borders made of 5 plants to be isolated. A total of 15 homogeneous plots of land were counted. A completely randomized design with 5 treatments and 3 repetitions was used for this trial (plots of land). The efficacy of these treatments related to the aphids and their parasitoids through botanical formulations

was determined based on successive direct count (pepper leaves) to record the following variables: (a) the number of wingless adults and nymphs of aphids found on pepper leaves and (b) the number of parasitized aphids (mummies) on pepper leaves. In order to estimate the population density of aphids in pepper crops, the total number of aphids (wingless adults + nymphs) was observed on the underside of 4 leaves in the middle part of the plant out of 5 plants randomly selected in each plot. Additionally, 20 leaves with mummies were collected from each plot. Leaves from the middle part of the plant were randomly selected to directly count the number of mummies on each leaf. These mummies were observed in the laboratory through a stereostopic magnifier of 10 to 40X to obtain information about parasitism (Polack and Mitidieri, 2005).

Once the results were recorder, a mixed lineal model and a repeated measures ANOVA were applied. The treatment factor were considered the fixed effect and the monitoring date, the random effect. Considering that the response variable (number of aphids) did not fit a normal distribution, the square root transformation was applied. Since data exploration detected distint variability between sampling dates and treatments, the model included heterogeneous variances for different dates. In addition, LSD Fisher test was applied in order to compare the means of the treatments and a 5% significance level ($\alpha=0.05$) was set. In order to study the association between the number of aphids and the number of parasitized aphids per leaf, the Spearman rank correlation coefficient was used. The adjustment of model and the related hypothesis test were performed using the software InfoStat Profesional version 2014.

RESULTS AND DISCUSSION

The aphid was the pest observed in these monitoring processes. Figure 1 illustrates the mean number of aphids (wingless adults + nymphs) per leaf on different monitoring dates for each treatment.

The aphids recorded were *M. persicae* and *A. gossypii*, found in the crop since March 31st, 2016. Prior to the trial, it was observed that the mean number of aphids/leaf count was above the damage threshold. That is, more than 8 aphids (nymphs + adults) per leaf in some plots of land in different treatments (Figure 1). This situation demanded a control treatment with neem and potassium soap. Since then, the subsequent sampling dates only showed a subtle increase in the population of aphids per leaf after the application of two treatments (lecithin and control) whereas the other treatments remained with a number of aphids significantly lower than the damage threshold. Closer to the sampling

date of October 13th, the treatments (lecithin and control) recorded the highest number of the population of aphids. In the control treatment, the population exceeded the damage threshold (15.08 mean aphids per leaf), and in the second treatment, it yielded similar values (7.02 mean aphids per leaf). Under these circumstances, a biorational product needed to be applied. Subsequently, despite the fact that this application reduced the number of aphids per leaf, on the sampling date of October 17th, it was observed that the population in the control treatment increased again (5.17 mean aphids per leaf) as well as in the lecithin treatment (5.90 mean aphids per leaf), which was close to the damage threshold. However, in this case, it was decided not to apply any biorational product. On the subsequent sampling date of November 1st, it was observed that only in the control treatment the number of aphids increased to almost the damage threshold (5.62 mean aphids per leaf).

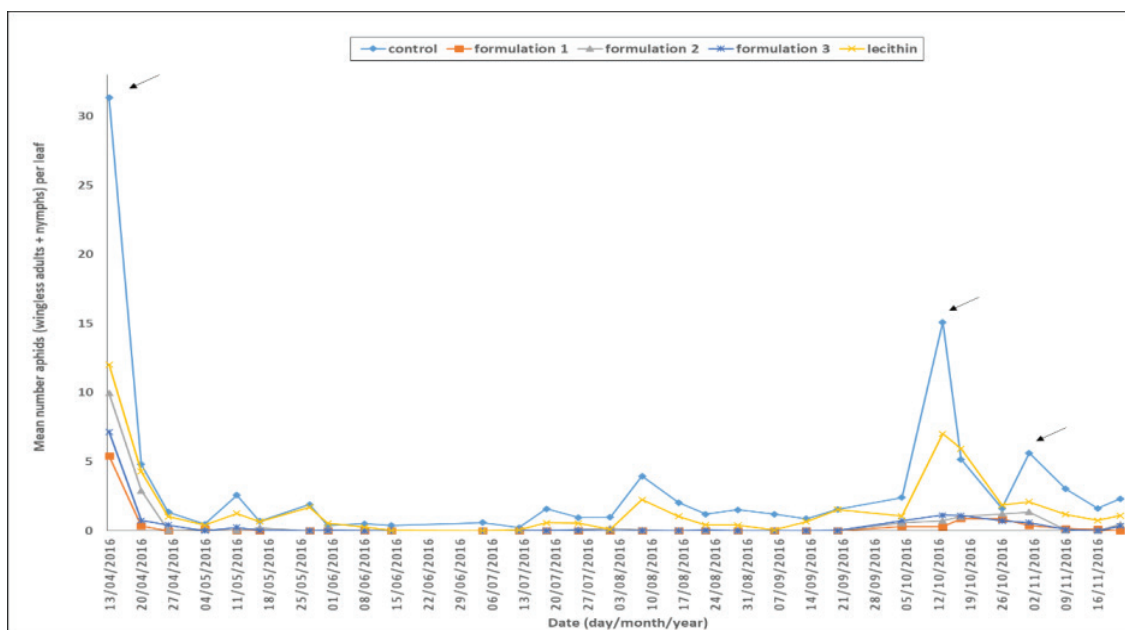


Figure 1. Aphids (nymphs + adults) per pepper leaf during the trial. The dates refer to the moments when a control treatment with biorational products was applied.

Thus, a control treatment was applied again. It is worth mentioning that the products were applied to each plot of land on the same date during the whole period of the trial. Concerning the treatments, the formulations always kept the plants with an extremely low number of aphids per leaf. Fixed effect of treatments was significant ($p < 0.001$), showing differences between mean number of aphids per treatment, which is clearly illustrated in Figure 1. Significant differences between means are shown in Figure 2A. The control treatment recorded the highest mean number of aphids during the period of the trial, followed by the lecithin and formulation 2. Finally, formulation 3 and formulation 1 showed a significant lower mean number of aphids.

In the trial, parasitoids *A. colemani* and *D. rapae* R. Paz, (personal communication, april 4, 2017) were identified. Regarding parasitism, it can be ascertained that a sparse population was found during the period of the trial because of its small number and low frequency of occurrence (Figure 3).

Notwithstanding the above, an increase in the parasitized aphids was observed during three sampling dates: August 16th (1.84 mean parasitized aphids per leaf), October 26th (5.73 mean parasitized aphids per leaf) and November 1st (2.47 mean parasitized aphids per leaf). These sampling dates correlated with the dates on which the highest number of aphids in the control treatment was recorded. In this regard, the correlation coefficient between the number of aphids and the number of parasitized aphids per leaf was 0.347 ($p < 0.0001$), which reveals a statistical association between these two variables. The results from the ANOVA for parasitized aphids showed significant differences among treatments ($p < 0.001$). Significant differences among the means of the treatments can be seen in Figure 2 B. It could be found that in the control treatment, the number of parasitized aphids per leaf was significantly higher than the other treatments, followed by the lecithin treatment. Finally, formulation 2, formulation 1, and formulation 3 were the treatments with lower number of parasitized aphids.

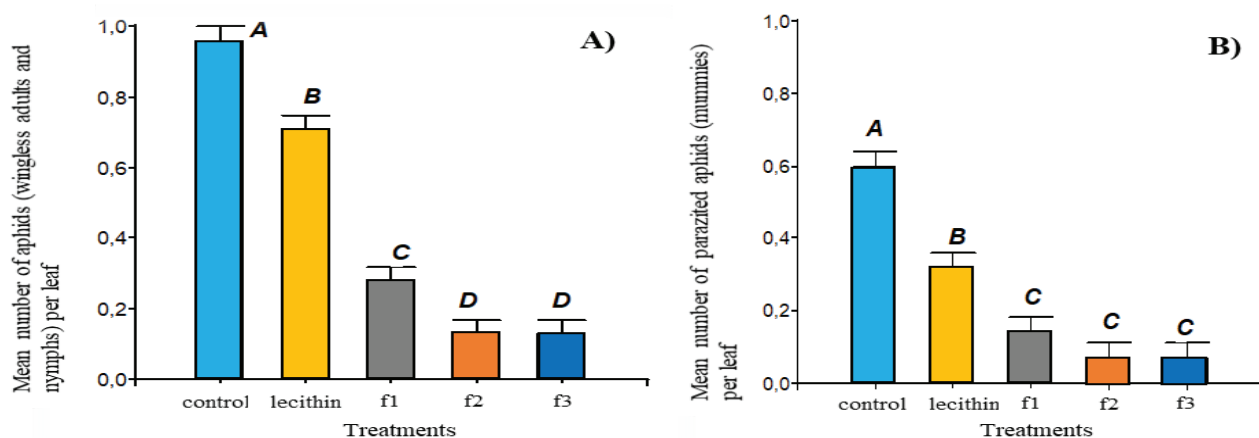


Figure 2. Estimated mean values of the square root of **A)** number of aphids and **B)** number of parasitized aphids per leaf in each treatment. Mean values with different letters indicate significant differences among the treatments (LSD, $p < 0.05$).

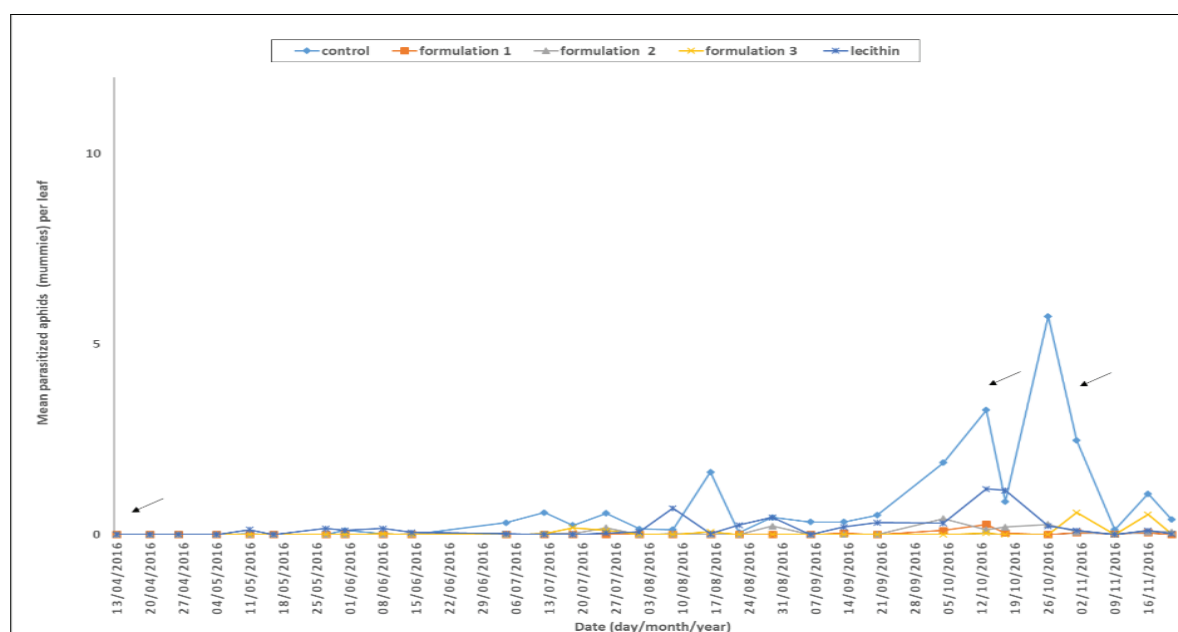


Figure 3. Parasitized aphids (mummies) per pepper leaf during the period of the trial. The date indicates the time when a control treatment with biorational products was applied.

In relation to the evolution of the population of aphids, the results observed in this study indicate that formulation 1 and 3 recorded a significantly lower mean number of aphids than the other treatments, as shown in Figure 1. This result was mainly the consequence of a remarkable repellent action, which could be confirmed by the absence of remains of dead aphids on leaves. On the other hand, it was seen that the plants treated with formulations derived from EOs constantly showed fewer aphids (adults and nymphs) compared to the control treatment, which suggests a repellent effect deriving from EOs.

Even though all EOs and Es are by-products of the plant metabolism, and plants use them to protect themselves from insects and mite pests which feed on them (Goyal *et al.*, 2012), their natural components, in isolation or combined with other components, may have biological activity that causes varied effects

such as contact insecticides, growth regulator, repellent, and anti-feedant activity (Mossa, 2016). Regarding the repellent activity, it is still very hard to identify the molecules that give rise to such effects as a result of natural active principles of EOs and Es (Isman, 2006). The application of formulation 1 composed of EOs of neem (*Azadirachta indica* A. Juss, *Meliaceae*), cinnamon (*Cinnamomum verum* J. Presl, *Lauraceae*), clove (*Syzygium aromaticum* L., *Myrtaceae*), oregano (*Origanum vulgare* L., *Lamiaceae*) and American marigold E (*Tagetes erecta* L., *Asteraceae*) showed a deterrent effect at the applied dose (5ml/litre of water), which might have resulted from the chemical components deriving from extracts and essential oils with repellent, anti-feedant, or insecticidal effects. These results were confirmed by studies conducted by Chaudhary *et al.* (2017), which indicated that one of the components of neem EO is azadirachtin, a tetranortriterpenoid

that causes effects on insect pest, including aphids.

Among these effects, feeding suppression, growth failure, and fecundity problems in the peach aphid could be found (Sulzer). Regarding the second component of formulation 1, cinnamon EO, Cheng *et al.* (2009) demonstrated that the chemical components of EOs of leaves of six species of *Cinnamomum* possess a larvicidal effect on different mosquitoes (*Aedes albopictus*, *Culex quinquefasciatus* and *Armigeres sulbatatus*) and reported a significant mortality after 24h and 48h. This effect is the result of the main component known as trans-cinnamaldehyde found in cinnamon EO (Prajapati *et al.*, 2005). Other previous studies, which included trans-cinnamaldehyde derived from cinnamon EO made by Hilje (2001) affirmed that a minimum dose of 0.1% (v/v) on *Bemisia tabaci* adults resulted in anti-feedant and oviposition deterrent effects. The third component of formulation 1 is clove EO, which has eugenol as its main component (Tian *et al.*, 2015), and it is considered as an insecticide by Jumbo *et al.* (2014).

In addition, previous studies conducted in the laboratory by Isman (2000) revealed that the mixture of thymol and eugenol applied to leaves modified the behaviour of nymphs of the second developmental stage of the peach aphid, since it was observed that a rate lower than 50% of aphids fed on the plant whereas the other aphids walked or were dead. As regards oregano EO, the biological activity and chemical composition has been studied by Xie *et al.* (2019), who confirmed that the main phenolic monoterpeneoid are carvacrol and thymol while other components can be found in less concentration such as *p*-cymen and γ - terpinene. The concentrations of these

various compounds depend on the area where this plant is cultivated and harvested (Faleiro *et al.*, 2003).

Additionally, recent studies made by Szczepanik *et al.* (2018) showed an effective control on the insects which affect stored products. Zhang *et al.* (2016) observed that the carvacrol compound had the most toxic effect on *Drosophila melanogaster*, and according to Park *et al.* (2016), especially on larvae and adults of fruit fly (*Drosophila suzukii*). Finally, formulation 1 contained American marigold EO (*Tagetes erecta* L., *Asteraceae*). Its flowers and leaves have a biocidal effect due to a wide array of secondary metabolites, including acyclic, monocyclic and bicyclic monoterpenes, sesquiterpenes, flavonoids, carotenoids and thiophenoids (Wanzala and Ogoma, 2013). The group of terpenoids includes Z- β -ocimene and dihydrotageton with nematicide activity in the egg and juveniles of nematode (*Meloidogyne incognita*). It should be noted that dihydrotageton is the highest toxic component (Adekunle *et al.*, 2007). In addition, Daedouri *et al.* (2019) determined that ethyl alcohol solutions of *Tagetes minuta* have a repellent and insecticidal effects as a result of their components deriving from bithienyl and Alpha-terthienyl. Therefore, the reduce number of aphids alive on pepper leaves resulted from the repellent effect of the natural substances applied on these plants.

The application of formulation 3 composed of EO of neem (*A. indica*), E of garlic (*Allium sativum* L. *Amaryllidaceae*), and E of rue (*Ruta graveolans* L., *Rutaceae*) gave rise to a repellent effect when a dose of 5ml/litre was applied. This effect was thought to be the result of various chemical components which are part of these Es and EOs. The E of garlic provides a high insecticidal potential (Awais

et al., 2014) due to its main sulfur components of higher biological activity such as allicin and diallyl trisulfide (Hrbek *et al.*, 2018), which are produced by cell decomposition (Awais *et al.*, 2014). In addition, the E of garlic was studied by Ho *et al.* (1996), who concluded that it produces a behaviour change in aphids *Myzus persicae* at the time they settle on the host plant due to its anti-feedant effect resulting in the death of aphids. Barati *et al.* (2014) showed that the E of garlic enhanced the reduction of oviposition of white flies in 56.6 and 56.5%, respectively, compared to the untreated controls. In other studies, Prowse *et al.* (2006) observed that the sulfur components of the E of garlic after contact with soil decompose, and consequently, are hydrolyzed resulting in a variety of isothiocyanates offering an insecticidal, nematocidal, and fungicidal effect.

Regarding the last component of this formulation, rue E, as well as other plants, it has active principles such as glucoside, flavonoid (rutin), and inulin (Haddouchi *et al.*, 2013), which have a repellent, nematocidal (larvicidal and adulticidal) as well as an insecticidal effect on various pests (Tavares *et al.*, 2013). In summary, the treatments formulation 1 and 3 displayed the same behaviour and recorded alive aphids on pepper leaves below the damage threshold. With respect to mummies per leaf, it could be observed that the mean of parasitized aphids significantly differed among treatments ($p < 0.001$). In plants in the control treatment, the count of aphids per leaf was significantly higher than the other treatments (Figure 2B). On the other hand, the lecithin treatment revealed a significantly higher mean of mummies than that observed in any of the other formulations. In relation to the control treatment (water spray), no considerable

repellent effect on aphids or their parasitoids on leaves was observed; therefore, a higher number of mummies was noticed.

These results correlate with those obtained by Reddy and Guerreo (2004), who studied volatile compounds emitted by plants injured by herbivore insects which promote the attraction of one or more specific natural enemies as a defence mechanism. Similarly, in the soy lecithin treatment, a number of aphids and mummies higher than the other treatments was reported; however, this number was lower than the one collected in the control treatment. These results might be attributed to the quality, as mentioned by Orthoefer (1980), of the soy lecithin to enhance the dispersing properties in cold water that form stable emulsions. On the other hand, it should be noted that this product added to ethyl lactate, propylenglicol, or carbitols can be used as insecticide, leather dyeing and tanning according to Jordan (1939). Thus, it could be inferred that the application of lecithin in the plots of land might have produced repellent effects, at a certain extent, on parasitoids resulting in a search behavior change among aphid colonies. Lastly, it should be mentioned that during the period of this trial, a low mean number of parasitized aphids was recorded.

CONCLUSIONS

The results of this trial showed that formulation 1 and formulation 3 have kept the number of aphids below the damage threshold, and in every treatment - either before or after the applications - aphid parasitoids spontaneously occurred. Notwithstanding this, it should be noted that the control and the lecithin treatment reported the highest number of mummies due to a higher number

of aphids. Therefore, we can conclude that the applications of different formulations based on botanical components, EOs and Es could be an interesting alternative to be applied in an integrated pest management (IPM) in greenhouse pepper crops. The use of these formulations derived from EOs and Es would considerably reduce the application of synthetic pesticides and allow an efficient aphid control resulting in a lesser impact on entomological fauna due to few residual effects, and hence promoting the development of a commercial sustainable product that would benefit the environment tremendously.

Conflict of interests: The authors declare that there is no conflict of interest.

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Coffee (*Coffea arabica* L, var. Castillo) seedling growth in Nariño, Colombia

Crecimiento en almácigo de café (*Coffea arabica* L, var. Castillo) en Nariño, Colombia

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ARTICLE DATA

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ABSTRACT

Coffee is one of the most socioeconomically important crops worldwide. Currently, environmental variations due to climate change are affecting coffee development and growth. Therefore, it is important to evaluate areas that offer a different environment during seedling stage since it is fundamental for successful cultivation. Four municipalities located at different altitudes in the Department of Nariño were selected: La Florida (1879 m a.s.l.), Sandoná (1924 m a.s.l.), Consacá (2001 m a.s.l.), and La Unión (1417 m a.s.l.). The experiment was conducted as a randomized complete block design. Growth variables were measured for five months, including total height-TH, number of leaves-NL, total leaf area-LA, fresh weight-FW, and dry weight-W. The following indices were calculated from the variables recorded: relative growth rate-RGR, leaf area index-LAI, net assimilation rate-NAR, crop growth rate-CGR, leaf area duration-LAD, and leaf area ratio-LAR. The data related to TH, NL, W, and LA recorded during the last evaluation (120 days after being transplanted) showed differences between locations; the maximum values in TH, W and LA were recorded in La Unión, while higher averages in NL were obtained in Sandoná. The index values for CGR, LAI, LAR, RGR, and LAD were higher in La Unión, while NAR was higher in La Florida. The results lead to conclude that there are differences in coffee seedling growth depending on the climatic conditions. Accordingly, the highest growth was observed in areas at lower altitudes; therefore, these are recommended for seedling establishment given the higher growth rate, especially that of leaves.

Keywords: coffee; environment; development; growth rates; seedlings.

RESUMEN

El café es uno de los cultivos de mayor importancia socioeconómica alrededor del mundo. Actualmente las variaciones ambientales debidas al cambio climático afectan su desarrollo y crecimiento. Siendo importante evaluar zonas con diferentes ofertas ambientales durante la etapa de almacigo, considerando que es la base para el éxito de un cultivo de café.

Se seleccionaron cuatro municipios en el departamento de Nariño a diferentes altitudes: La Florida (1879msnm), Sandoná (1924msnm), Consacá (2001msnm) y La Unión (1417msnm). El experimento se realizó en un diseño de bloques completos al azar. Durante cinco meses, se registraron variables de crecimiento: altura total-AT, número de hojas-NH, área foliar total-AF, masa fresca-WF y masa seca-W. A partir de estas se calcularon índices derivados: tasa relativa de crecimiento-TRC, índice de área foliar-IAF, tasa de asimilación neta-TAN, tasa de crecimiento del cultivo-TCC, duración de área foliar-DAF y relación de área foliar-RAF. Los datos de AT, NH, W y AF registrados en la última evaluación (120 días después del trasplante) muestran diferencias entre localidades. Los valores máximos en AT, W y AF se presentaron en La Unión, mientras que los promedios superiores en NH se obtuvieron en Sandoná. Las variables TCC, IAF, RAF, TRC y DAF presentaron mayores índices en La Unión, mientras que TAN en La Florida. Concluyendo que hay diferencias en el crecimiento de plántulas de café según las condiciones climáticas donde se estén desarrollando, por cuanto se recomiendan localidades con menor altitud para el establecimiento de almácigos dado el mayor crecimiento obtenido, siendo las hojas la sección de mayor influencia.

Palabras clave: café; ambiente; desarrollo; índices de crecimiento; plántulas.

INTRODUCTION

Coffee (*Coffea arabica* L) cultivation is a highly profitable economic activity in several Latin American countries. Furthermore, it determines the subsistence of several sectors of the population given the diverse activities and income that are generated across the value chain (i.e., from cultivation to consumption) (Bucardo *et al.*, 2016; García *et al.*, 2017; Robiglio *et al.*, 2017; Gil, 2019; Molina *et al.*, 2020).

In Colombia, coffee production occupied nearly 742.3 thousand hectares in 2018, with a production of 855 thousand tons. Specifically, 4.51% of the crop area (33.465 ha) and 4.17% of the production (35.679 tons) were provided by the Department of Nariño. This coffee-growing area is located near the equator at altitudes ranging from 1200 to 2300 m a.s.l., covering a wide range of production systems (Agronet, 2020; Ordóñez *et al.*, 2018).

Given the broad socioeconomic importance and extensive cultivation of coffee, Government and non-governmental organizations (NGOs)

must focus on addressing the impact of climate conditions on coffee growth, production, and pest and diseases (Bucardo *et al.*, 2016; García *et al.*, 2017; Robiglio *et al.*, 2017; Gil, 2019; Molina *et al.*, 2020).

Currently, and as predicted for the XXI century, climate change is affecting different crops, including coffee (García *et al.*, 2017; Alfonse *et al.*, 2018). This is due to weather variability, which directly affects perennial crop growth and development (Farfán, 2016).

Several environmental factors (e.g., climatic, edaphic, and biotic) may affect coffee growth, development, and physiological processes. For instance, high altitudes favor the concentration of elements in rubbish dump. Furthermore, climatic factors, such as light intensity or radiation, precipitation, temperature, and CO₂, determine which areas are ecologically suitable for coffee crop development. However, in regions with marked dry periods, the response of coffee is more influenced by precipitation or soil humidity, while in regions without soil water deficit, coffee response is related to radiation and the phenological stage of the

crop (Melo and Piñeros, 2015; Rodríguez *et al.*, 2015; Robiglio *et al.*, 2017; García *et al.*, 2017; Borjass *et al.*, 2018; Milla *et al.*, 2019; Vega *et al.*, 2020).

As an adaptive strategy of the coffee production industry to climatic variability, the Federación Nacional de Cafeteros of Colombia (National Federation of Coffee Growers of Colombia) proposes planting improved varieties, renovating coffee plantations, and conducting research focused on climate change (Farfán, 2016). Furthermore, detailed studies at the farm and landscape levels are necessary to determine how the climate affects the different phenological stages of the crop (Molina *et al.*, 2020). This highlights the importance of conducting research on seedlings of coffee Castillo variety to propose solutions in the face of climate change.

In this sense, this research aimed to evaluate the growth of *C. arabica* Castillo variety at different altitudes in the municipalities of La Florida, Sandoná, Consacá, and La Unión in the Department of Nariño. This study contributes knowledge to optimize resources and aid in decision-making when it comes to coffee seedling management.

MATERIALS AND METHODS

Location. This study was conducted during the first semester of 2014 in four municipalities of the Department of Nariño: La Florida *vereda* Santana at 1846 m a.s.l., Sandoná *vereda* San Isidro at 1880 m a.s.l., Consaca *vereda* San Antonio at 2001 m a.s.l., and La Unión *vereda* La Playa at 1417 m a.s.l., under the climatic conditions described in Table 1.

Table 1. Climatic Parameters of the study areas for seedling establishment in four municipalities of the Department of Nariño- Colombia: La Unión, Sandoná, La Florida, and Consacá.

Bioclimatic Variable	La Unión	Sandoná	La Florida	Consacá
Mean temperature (°C)	20.8	17.7	18	16
Mean daily temperature (maxtemp – mintemp; °C)	10.5	10.2	10.2	10
Maximum temperature of the hottest month (Tmax; °C)	27.5	23.6	24.1	21.6
Minimum temperature of the coldest month (Tmin; °C)	15.2	12.2	12.4	10.5
Thermal amplitude (Tmax– Tmin; °C)	12.3	11.4	11.7	11.1
Mean temperature of the most humid trimester (°C)	20.3	17.4	17.6	15.9
Mean temperature of the driest trimester (°C)	21.2	17.8	18.2	16
Solar brightness (wat/m ²)	529.91	351.71	444.07	322.75
Photosynthetically Active Radiation (μM/m ² s) (PAR)	1049.16	720.82	902.96	659.47

The evaluation of the variables was done at the laboratory of Plant Physiology of the Faculty of Agricultural Sciences at Universidad de Nariño, Pasto.

Plant material. In the four municipalities, Coffee Castillo “Tambo” variety was planted in flat seed beds with guadua (*Guadua angustifolia* Kunth), in an area of 1.5 m² per kilogram of planted seed. The substrate was washed river sand (0.30m thick) that was disinfected with hot water (90°C). After an average of 90 days, “chapola” plants (i.e., coffee plant stage in which cotyledon leaves are noticeable) were obtained and transplanted in 1 Kg bags filled with a mix of sand and organic matter (3:1). The seedlings were covered with 30% polyshade and agronomic management was done according to the recommendations from Cenicafé and Federación Nacional de Cafeteros de Colombia (2013).

Experimental design. A randomized complete block design was used, in which the blocking factor corresponded to the altitude and climate of the sites where the study seedlings were located.

Variables evaluated. Evaluations were carried out every 15 days on 20 randomly selected seedlings for a total of nine evaluations. The total height (TH) was measured from the base of the stem to the

apex with a ruler in centimeters and the number of leaves (NL) was based on the direct count.

Plant material sampling. In each evaluation, two seedlings were randomly extracted to evaluate the following variables in the laboratory: total leaf area (LA) in square centimeters by applying the leaf length-dependent model, where $LA = \left(\frac{4.541 + (2.38 * TH)}{1 + (0.154 * TH) + (0.0075 * TH^2)} \right) * L$; fresh weight (FW) was determined through direct weighing, and dry weight (W) was estimated by oven-drying each organ (e.g., stem, leaves, and roots) at 60°C for 72h to quantify the total W and W of each fraction, in grams (g).

The variables measured were used to calculate indices such as the relative growth rate (RGR), leaf area index (LAI), net assimilation rate (NAR), crop growth rate (CGR), leaf area duration (LAD), and leaf area ratio (LAR), according to the equations shown in Table 2 (Gardner *et al.*, 1985).

Data analysis. The data obtained for each variable were consolidated in an Excel spreadsheet and analyzed based on the Functional Analysis of Growth methodology, which uses measurements based on frequent time intervals with a small number of plants, using a regression model (Gardner *et al.*, 1985).

Table 2. Components of growth parameters determined in coffee Castillo “Tambo” variety seedlings.

Growth indices	Abbreviation	Equation for mean value	Units
Relative Growth Rate	RGR	$\frac{\ln W_2 - \ln W_1}{T_2 - T_1}$	$Weight * Weight^{-1} * Time^{-1}$ $g * g^{-1} * d^{-1}$
Net Assimilation Rate	NAR	$\frac{W_2 - W_1}{T_2 - T_1} * \frac{\ln LA_2 - \ln LA_1}{LA_2 - LA_1}$	$Weight * Area^{-1} * Time^{-1}$ $g * cm^{-2} * d^{-1}$
Leaf Area Index	LAI	$\frac{LA_2 - LA_1}{As}$	Adimensional
Leaf Area Ratio	LAR	$\frac{\left(\frac{LA_1}{W_1}\right) + \left(\frac{LA_2}{W_2}\right)}{2}$	$Area * Weight^{-1}$ $cm^2 * d^{-1}$
Crop Growth Rate	CGR	$\frac{1}{As} * \frac{W_2 - W_1}{T_2 - T_1}$	$Weight * Area^{-1} * Time^{-1}$ $g * cm^{-2} * d^{-1}$
Leaf Area Duration	LAD	$\frac{(LAI_1 + LAI_2)(T_2 - T_1)}{2}$	Time d

Symbols used: W=Total Dry Weight; T=Time; LA=Leaf Area; As=Soil area; ln=Natural Logarithm.

RESULTS AND DISCUSSION

In the four municipalities, TH increases over time (Figure 1). However, the seedlings in La Unión showed higher values since the first evaluation, reaching a height of 14.5 cm. The TH of seedlings in Consacá, Sandoná, and La Florida were lower, as indicated by means of 8.28, 7.83, and 6.25cm, respectively. Borjas *et al.* (2018) report a height range similar to the one found here, specifically, between 7.56 and 13.89cm. However, these heights are lower than those reported by Álvarez (2019), who obtained an overall mean of 16.56cm.

The data recorded for TH in the municipalities of La Unión and La Florida displayed a second-degree polynomial behavior, while TH in Consacá and Sandoná adjusted to

a linear model (Figure 1). Furthermore, the predictive models for La Unión and La Florida vary according to the values obtained for the predictive variable, while in Consacá and Sandoná, these values are direct and constant.

Regarding temperature (Table 1), La Unión showed the highest values, thus promoting growth. Higher temperatures are associated with a higher photosynthetic assimilation rate, which is directly related to growth. Moreover, the effect of temperature is observed in the duration of the phenological stages. However, this study demonstrates that higher altitudes lead to reduced growth of the aerial part of the plant due to lower temperatures (Cenicafé and Federación Nacional de Cafeteros de Colombia, 2013; Gil, 2019).

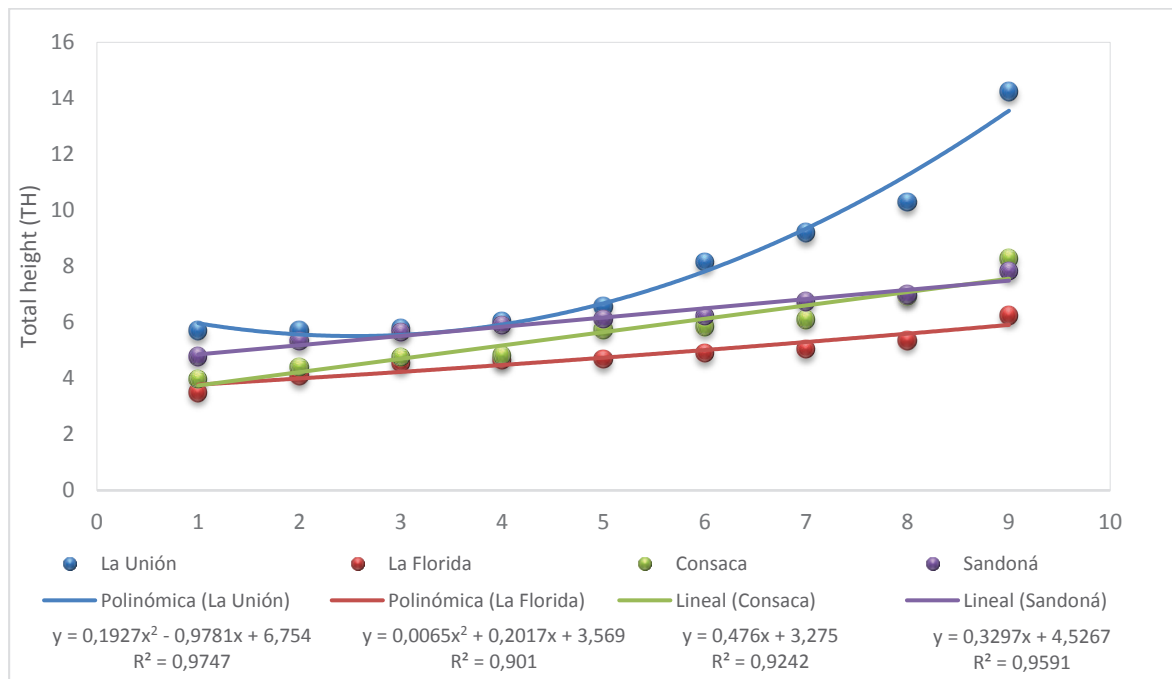


Figure 1. Total height of coffee seedlings evaluated in four coffee-growing municipalities in southern Colombia.

The rate of leaf emergence showed constant growth with mean values ranging from one to two leaves every 15 days (Figure 2). Leaf development is related to solar brightness and photosynthetically active radiation

since there is evidence that leaf formation is constant; furthermore, different degrees of shade generate more or a fewer number of leaves (Milla *et al.*, 2019).

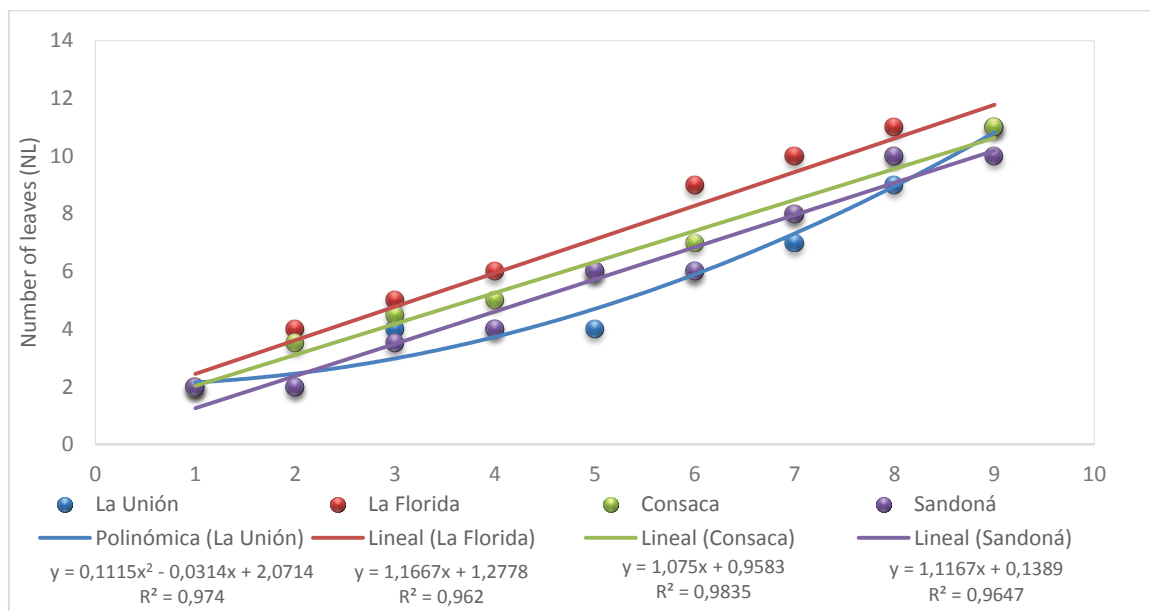


Figure 2. Number of leaves of coffee seedlings evaluated in four coffee-growing municipalities in southern Colombia.

The seedlings in the municipality of La Florida showed a higher and constant rate of leaf emergence across the nine evaluations, in contrast to the other municipalities where the same number of leaves was observed across successive evaluations. However, in the last evaluation, the plants in La Unión and Consacá showed 11 leaves each (Figure 2).

In this regard, Borjas *et al.* (2018) obtained a higher number of leaves (14 leaves) at the 60-day evaluation. The importance of this variable lies in the direct relationship between the number of leaves and leaf area, as well as between these two variables and growth. The leaf surface is responsible for carbon assimilation, nutrient and water use, evapotranspiration, development rate, and photosynthetic efficiency (Cenicafé and Federación Nacional de Cafeteros de Colombia, 2013; Montoya *et al.*, 2017; Gil, 2019).

In the four localities, the increase in NL was modeled by linear equations, except in La Unión, where it adjusted to a second-degree polynomial. The coefficients of determination were significant ($R^2 > 0.96$), indicating a good fit. According to Letort *et al.* (2020), there is an almost linear branching growth, reaching 14 knots with a pair of true leaves and stabilizing at a constant value of 1. This growth trend allows quantifying the increase in the number of leaves adjusted to a power function. In contrast, Borjas *et al.* (2018) obtained first and second-degree equations to model the increase in NL. However, in this research, only La Unión obtained results similar to reports in the literature, possibly due to a higher temperature, solar brightness, and radiation (Table 1) in this locality. Conversely, the other three municipalities showed lower mean values for these climatic variables.

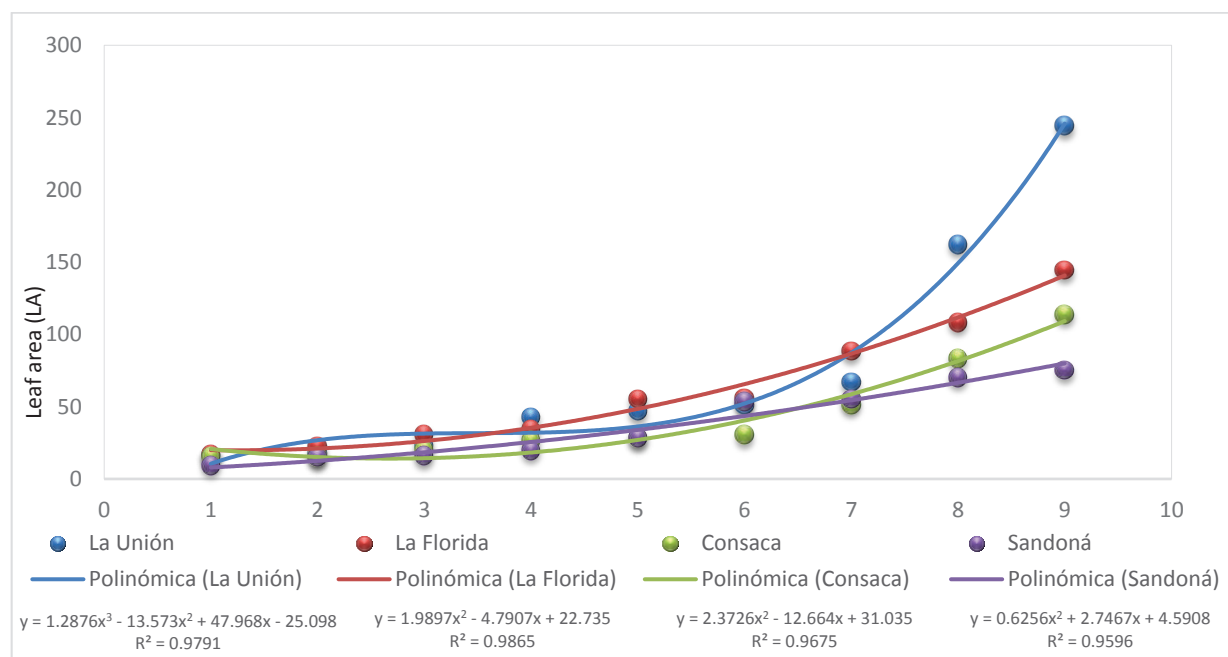


Figure 3. Leaf area of coffee seedlings evaluated in four coffee-growing municipalities in southern Colombia.

The highest mean value for LA was obtained in La Unión (244.05cm²), followed by La Florida (125.05cm²), Consacá (112.84cm²), and Sandoná (75.21cm²). However, the four municipalities showed an increase in LA over time that adjusted to a polynomial model (Figure 3). The model showed a good fit, indicated by an R² greater than 0.95 for the four localities, which demonstrated the validity of the growth indices derived from the LA variable.

Additionally, studies based on comparisons of leaf area across robusta and arabica coffee plants report significant increases between four- and seven-month periods in response to temperature (Vega *et al.*, 2020).

Regarding W, the seedlings from the municipality of La Unión reached a high mean dry weight (2.26g), followed by La Florida

(1.61g), Sandoná (1.23g), and Consacá (1.18g). Seedling development was explained by a third-degree polynomial for La Unión and a second-degree polynomial for the other municipalities. R² values ranging from 94 to 98% were obtained for the models (Figure 4). These results demonstrate the effect of temperature on the dry matter accumulation rate and growth of coffee seedlings (Cenicafé and Federación Nacional de Cafeteros de Colombia, 2013; Montoya *et al.*, 2017; Gil, 2019).

On the other hand, seedling growth, expressed as mean fresh weight, in the four localities, indicated that the leaves accounted for the highest percentage (65.88%) of the total weight, followed by the stem (32.06%) and roots (2.06%). However, in the last evaluations, root growth was greater than leaf and stem growth (Figure 5A).

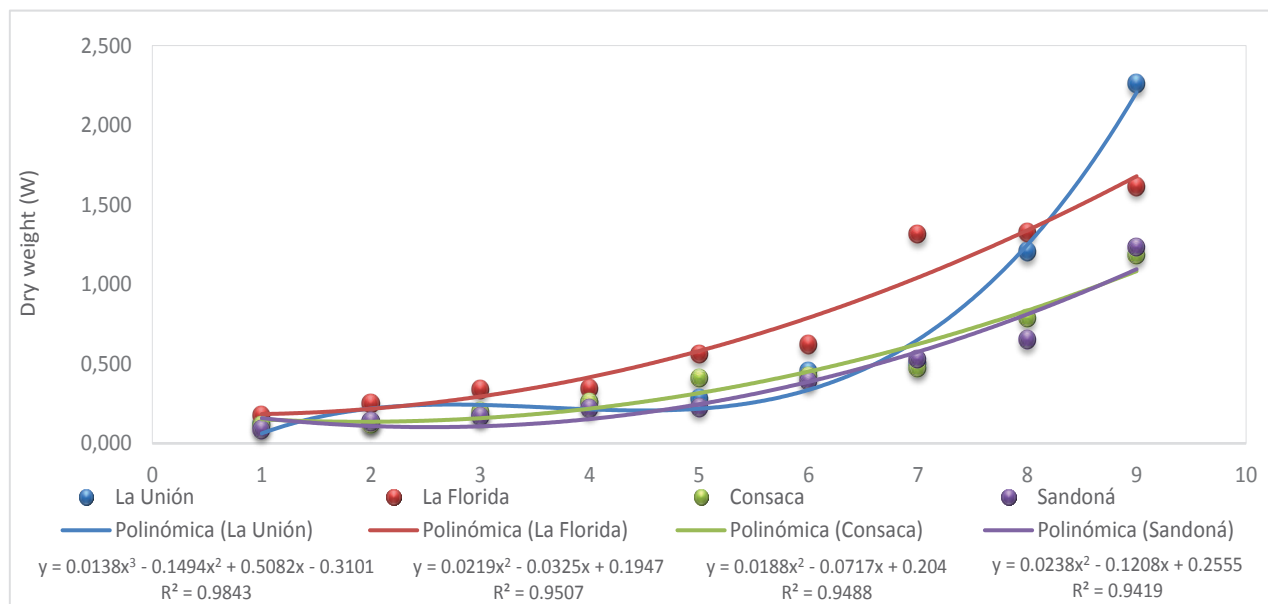


Figure 4. Dry weight of coffee seedlings evaluated in four coffee-growing municipalities in southern Colombia.

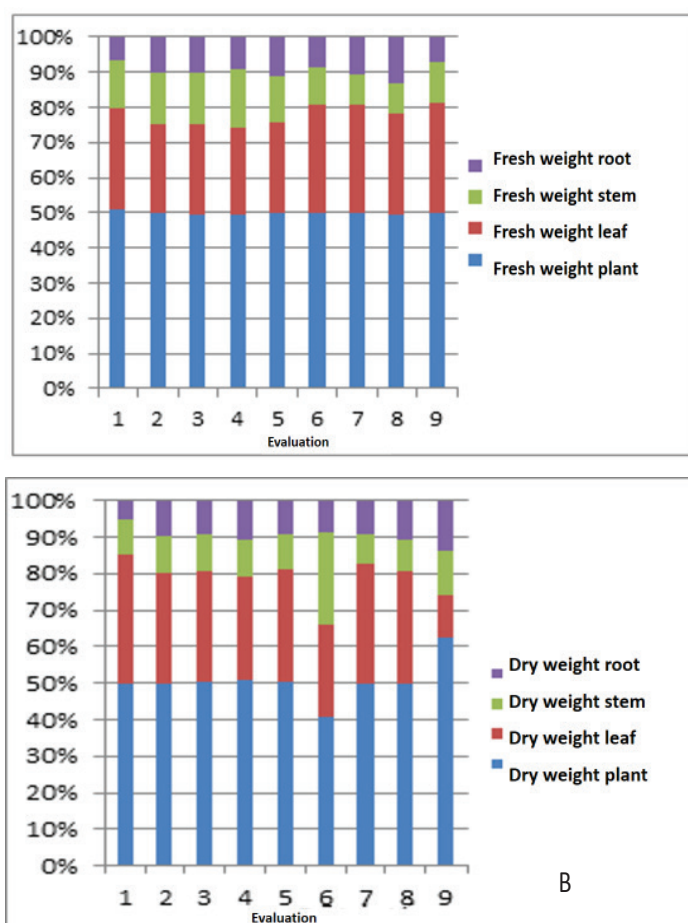


Figure 5. Distribution of fresh weight (A) and dry weight (B) of coffee (*Coffea arabica* L) Castillo variety seedlings over time in four localities.

Similar to the results found here, Borjas *et al.* (2018) reported greater biomass in the aerial part of the plant compared to the roots. However, there is a balance between the leaf area or total photosynthetic surface for the production of assimilates and the root surface area for absorption of water and minerals. Nonetheless, the root/aerial part ratio (R/AP) gradually decreases with age since there is higher accumulation of carbohydrates and root development. Additionally, CO₂ and temperature have significant effects on aerial biomass accumulation between four and seven months of development of arabica and robusta coffee. However, at 12 months,

arabica showed an increase in several plant parameters in response to CO₂, while robusta was unaffected by CO₂ (Vega *et al.*, 2020).

The dry weight of each plant part and its relationship with the total weight showed that 72.55% of the dry weight corresponded to the leaves (finding also observed for fresh weight), followed by the roots and stem (Figure 5B).

Given these findings, the stem has a high proportion of water and low dry matter, while the leaves are the main contributors to the total plant weight. These results are in

line with those of Encalada *et al.*, (2016), who report that the greatest contribution to the total dry weight was made by the leaves and roots. Moreover, as described for NL variable, the results demonstrate the effect of the temperature, as well as solar brightness and photosynthetically active radiation, on fresh and dry biomass. Similarly, other studies demonstrate that 20% light exposure promotes greater dry mass and specific leaf area (Cenicafé and Federación Nacional de Cafeteros de Colombia, 2013; Encalada *et al.*, 2016; Montoya *et al.*, 2017; Gil, 2019).

On average, in the seedling developmental stage of coffee Castillo variety, 83% of the plant is water and 17% is dry matter. Letort *et al.* (2020) describe a progressive increase in the relationship between offer and demand of biomass in seedlings, corresponding to the establishment of the plant architecture, represented by a gradual increase in organ size and the appearance of the first branches.

On the other hand, RGR varied across evaluations in the four localities, displaying the lowest dry matter accumulation between evaluations. The highest and lowest RGR were observed at the seventh and eighth evaluations in La Unión and La Florida, respectively (Figure 6A). Gil (2019) mentions that temperatures above 24°C accelerate plant growth compared with low temperatures that delay development.

The NAR also varied across all evaluations in the four municipalities; for instance, in La Florida, it reached a maximum value (0.00067g cm⁻²d⁻¹) at 105 days (Figure 6B). This behavior is explained by Rodríguez *et al.* (2015), who described that the leaf system of coffee trees exposed to high solar radiation showed lower sizes, greater histological

thickness of the palisade parenchyma, and higher stomatal frequency.

A lower number of leaves and leaf area exposed to radiation, concomitantly with higher NAR values, indicates that CO₂ assimilation is more efficient. The NAR is a measure of the net gain of assimilates per unit leaf area unit and unit time (Gardner *et al.*, 1985).

The maximum LAI was obtained at the eighth and ninth evaluations in the municipality of La Unión, while the lowest value (0.39) was obtained at the same evaluation periods in Sandoná (Figure 6C). The LAI varies according to the size, number, and vertical and horizontal distributions of the foliage. Therefore, leaf growth parameters in coffee seedlings are determined by leaf supporter axis order and position along the vertical profile, as well as duration of leaf expansion. Accordingly, these parameters suggest the effects of age and micro-environmental light modulation on growth (Rakocevic and Takeshi, 2018).

For LAR, the highest values were obtained at 75 days (fifth evaluation) in La Unión, at 60 days in La Florida, and 30 days in Sandoná and Consacá (157.5, 96.3, 104.03, and 130.8cm²*d⁻¹, respectively) (Figure 6D). These findings suggest a maximum photosynthetic capacity and low respiratory cost since almost all leaves are exposed to the LAR during the seedling stage. Furthermore, plants use a greater fraction of photoassimilates for the growth and development of photosynthetically active areas, thus, generating energy expenditure that leads to lower weight (Cenicafé and Federación Nacional de Cafeteros de Colombia, 2013).

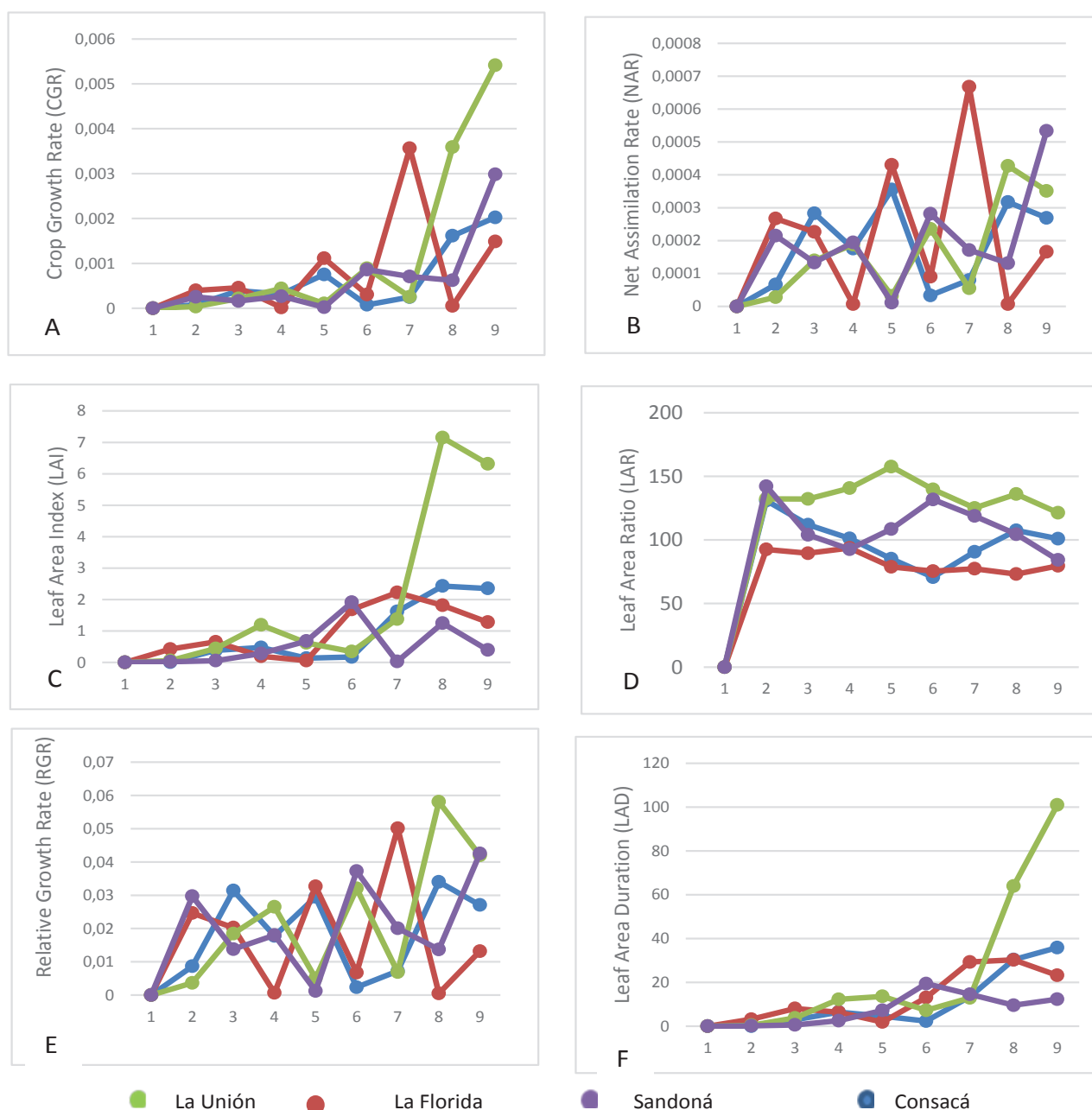


Figure 6. Growth indices of coffee seedlings in the municipalities of Consacá, Sondoná, La Unión, and La Florida: A) Relative Growth Rate, B) Net Assimilation Rate, C) Leaf Area Index, D) Leaf Area Ratio, E) Crop Growth Rate, and F) Leaf Area Duration.

Crop growth rate (CGR) under the environmental conditions of the four localities was low until the seventh evaluation (105 days). From this moment, the plants in La Unión, Sondoná, and Consacá showed a progressive increase, reaching maximum

values at 135 days, as indicated by mean CGRs of $0.0054\text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ in La Unión, $0.0029\text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ in Sondoná, and $0.0020\text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ in Consacá. In La Florida, the greatest dry biomass accumulation per unit of soil area ($0.0035\text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$) was obtained at 105 days.

The results show that the maximum mean CGR values in the four localities were reached at the last evaluations. These findings agree with those of Letort *et al.* (2020), who mention that biomass production per unit of time displays a sigmoid trend, considering the saturation generated by the auto-shading effect of the leaves as the plant grows.

The maximum LAD was observed in plants in the municipality of La Unión at the last evaluation (135 days), followed by the plants in Consacá, La Florida, and Sandoná (100.9, 35.8, 23.2, and 1.3 days, respectively). This index is associated with LAI over time and represents leaf production capacity during crop growth. LAD is also related to the dramatic effect of the first leaf drop on biomass production, which depends on several factors such as the environment and phenological stage (Cenicafé and Federación Nacional de Cafeteros de Colombia, 2013; Letort *et al.*, 2020).

CONCLUSIONS

The locality in La Unión, situated at lower altitude with higher temperature, showed the highest means for the direct variables and derived indices.

In all localities, the stems displayed the lowest fresh and dry weights in response to the progressive growth of the aerial parts of the plant and the highest accumulation of dry matter in the leaves. This is explained by the fact that leaf area has the greatest effect on coffee plant weight during seedling stage.

The temperature conditions are widely associated with the altitude of the localities in this study and directly affect the variables

analyzed. Therefore, at higher altitude, plant growth, dry weight of the aerial part, number of leaves per plant, and dry weight are lower.

Conflict of interest: The authors declare that there is no conflict of interest.

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