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Determination of *Aloysia citriodora* (Palau) phenology and the associated arthropods

Determinación de la fenología de *Aloysia citriodora* (Palau) y los artrópodos asociados

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Insects of the order Coleoptera, frequent visitors of lemon verbena plants.

Photo: S.P. López-Zapata

ABSTRACT

Lemon verbena, *Aloysia citriodora* (Palau), has been widely used in traditional medicine, cosmetics, and the food industry. In Colombia the foliage is marketed fresh, but there is no information regarding the plant phenology or the associated insects; therefore the management practices are still empirical and sometimes do not comply with the national regulations for the commercialization of medicinal plants. This study determined that it takes 32 weeks from planting to flower senescence to complete its phenological cycle; likewise, an associated arthropofauna of 50 families with different feeding habits were found, suggesting that the species offers different ecosystem services. The information obtained could contribute to establishing more effective management plans for arthropods and to improve quality of materials with medicinal purposes.

Additional key words: pest monitoring; medicinal plants; growth period; Verbenaceae.

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RESUMEN

El cidrón, *Aloysia citriodora* (Palau) ha sido ampliamente utilizada en la medicina tradicional, la cosmética y la industria alimentaria. En Colombia el follaje se comercializa fresco, pero no existe información sobre la fenología de la planta ni los insectos asociados; por lo que las prácticas de manejo son aún empíricas, y en ocasiones no cumplen con la norma nacional para la comercialización de plantas medicinales. Este estudio determinó que la planta tarda 32 semanas desde la siembra hasta la senescencia de la flor para completar su ciclo fenológico; asimismo, se encontró una artrofauna asociada de 50 familias con diferentes hábitos alimentarios, lo que sugiere que la especie ofrece diferentes servicios ecosistémicos. La información obtenida podría contribuir a establecer planes de manejo más efectivos de artrópodos y a mejorar la calidad de los materiales con fines medicinales.

Palabras clave: monitoreo de plagas; plantas medicinales; periodo de crecimiento; Verbenaceae.

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INTRODUCTION

The lemon verbena (*Aloysia citriodora* (Palau) or *Aloysia triphylla* [L'Hér.] B.) is native to South America, North Africa and Southern Europe (Bahramsoltani *et al.*, 2018). It belongs to the Verbenaceae family and possesses antimicrobial (Oliva *et al*., 2010), and antiviral properties (Meneses *et al*., 2009); it has been used in infusions to control fever, insomnia, anxiety, some heart problems, and gastrointestinal disorders (Argyropoulou *et al*., 2007). This utilization dates to the 17th century, due to the species' ethnopharmacological importance, in addition to other uses in the cosmetic and food industries (Bahramsoltani *et al.*, 2018).

The major components of the plant are geranial (27.3%), neral (22.5%), geraniol (6.2%), bicyclogermacrene (5.2%) and nerol (4.9%) (Rojas, *et al*., 2010). According to Stashenko *et al*. (2003) abundant citral, nerol, and geraniol, have been found in cultivated plants in Colombia; in addition, phenolic compounds, and flavonoids such as chrysoeriol, luteolin, and phenylpropanoid derivatives have been identified as active components (Leyva-Jiménez *et al*., 2018). These compounds have been successfully used in the *in vitro* treatment of bacteria (Rojas *et al*., 2010), viruses (Aurori *et al*., 2016), as antioxidants (Leyva-Jiménez *et al*., 2020), in diabetes treatments (Diez-Echave *et al*., 2020), in tumor prevention (Salama *et al*., 2021), and in fruit postharvest (Fontana *et al*., 2021; Shirzad *et al*., 2021).

Although the plant has been extensively studied for therapeutic and medicinal uses, as well as the composition of essential oils, there is no research related to its phenological stages or the insects associated with each stage in Colombia. The aim of the study is to address this gap of knowledge and to generate better agronomic practices for its cultivation in the country.

MATERIALS AND METHODS

The study was conducted at the El Jazmin farm, in the municipality of Santa Rosa de Cabal, Colombia, located at 1,650 m a.s.l, with a relative humidity of 78%, temperature of 20°C, annual rainfall of 2,620 mm, daily sunshine of 3.6 h, and wind speed of 4 km h^{-1} with a predominant west direction (data from the Veracruz station, IDEAM, 2023).

Cuts 15 cm long obtained from adult plants were rooted during 20 d before being transplanted to the field. The experimental unit was subdivided in 18 subplots of 15 plants each; the distance between plants was 0.5 m, and 1.0 m between rows. The terrain slope was 30%, with soils derived from volcanic ash with an organic matter content greater than 8%. Crop management was based on agroecological practices, including fertilization with organic matter and weed control using manual tools.

The modified BBCH scale (Arcila *et al*., 2001) was used to describe the phenological stages, establishing a uniform coding to describe growth stages. Only 7 stages were taken because the species does not fruit under local environmental conditions, and an additional stage corresponding to floral senescence was included. In the nursery and field phase, 10%

of the plants were evaluated weekly by measuring the branches. It was established as a parameter that 50% of them should show the morphology described in the scale, including the reproductive stage where flowers were open.

For the evaluation of the arthropofauna, the methodology proposed by Wolf-Echeverry (2006) was used, monitoring all the plants weekly and during the identified phenological stages, including swabbing at 20 cm from the soil surface. For small insects, pollinators, parasitoids and ants, suction bottles were used. Identification was made to the levels of order, family, and in some cases even genus, using taxonomic keys (Triplehorn and Johnson, 2005), with the help of specialists from the areas of Biology and Agronomy of the Universidad del Quindío and the Universidad de Caldas (Colombia). Finally, all specimens collected were deposited in the Entomological Collection of the Corporacion Universitaria Santa Rosa de Cabal - CUS-E (Insects) with the Registro Unico Nacional de Colecciones Biologicas (RNC) number 279 (December 15, 2021).

RESULTS AND DISCUSSION

Determination of phenological stages

During 32 weeks of evaluation, 7 growth stages were identified, 5 corresponding to the vegetative phase (Vo to V4), and 2 to the reproductive one (R1 and R2, Tab. 1). The V0 or nursery stage began with slow growth, developing shoots from the third week after sowing. This phase culminated with seedlings with an average of 5 roots and a length of 1.22 cm (Fig. 1). Under these conditions, the emission of shoots occurred between 2 and 3 weeks, and the emission of roots occurred between 6 and 8 weeks, for 95 and 73% of the seedlings evaluated, respectively.

The percentage of rooting in contrast to that obtained in shoots is explained by the difficulty of the species to form roots (Potocnjak, 2003), generating vegetative buds even in plants without root development. This behavior is caused by the reserves accumulated in the cutting, which cannot sustain the seedling in formation, subsequently causing the death of the shoots (Gaibor-Tulcanaz, 2016). This condition specifically in Colombia generates difficulty in the propagation of the species because the required environmental conditions are not present for seed production and natural defoliation, determining characteristics not only for growth but also for the synthesis of secondary metabolites (Aliniaeifard *et al*., 2010; El-Hawary *et al*., 2012).

Stage V1 occurred from week 5 to week 12 and was characterized by an increase in the number and length of primary branches, culminating in seedlings that presented an average of 5 primary branches and an average length of 13.2 cm. Stage V2 started from week 13 to week 16, during which a constant number

Table 1. BBCH scale for the description of *A. citriodora* phenological stages.

Main growth stage 0 (V0)	Vegetative propagation
00	Orthotropic or plagiotropic cuttings, with more than 3 knots, 15 cm long, without leaves
01	Seeded cutting, without sprouts or callus formation
03	Callus formation on cuttings. Rounded green buds. Visible on stems
05	Bud formation on the cutting
06	Root formation in the cutting
07	Formation of buds on cuttings, with 2 or 3 nodes and branched roots
09	Cuttings with roots 6 to 7 cm long and shoots with 4 to 5 nodes
Main growth stage 1 (V1)	Development of primary branches
Main growth stage 2 (V2)	Development of secondary branches, plants in the field
Main growth stage 3 (V3)	Elongation of primary and secondary branches, initiation of tertiary branch development
Main growth stage 4 (V4)	Elongation of primary, secondary and tertiary branches, and development of quaternary branches
Main growth stage 5 (R1)	Development of floral primordia and flower anthesis
Main growth stage 6 (R2)	Flowering and flower senescence

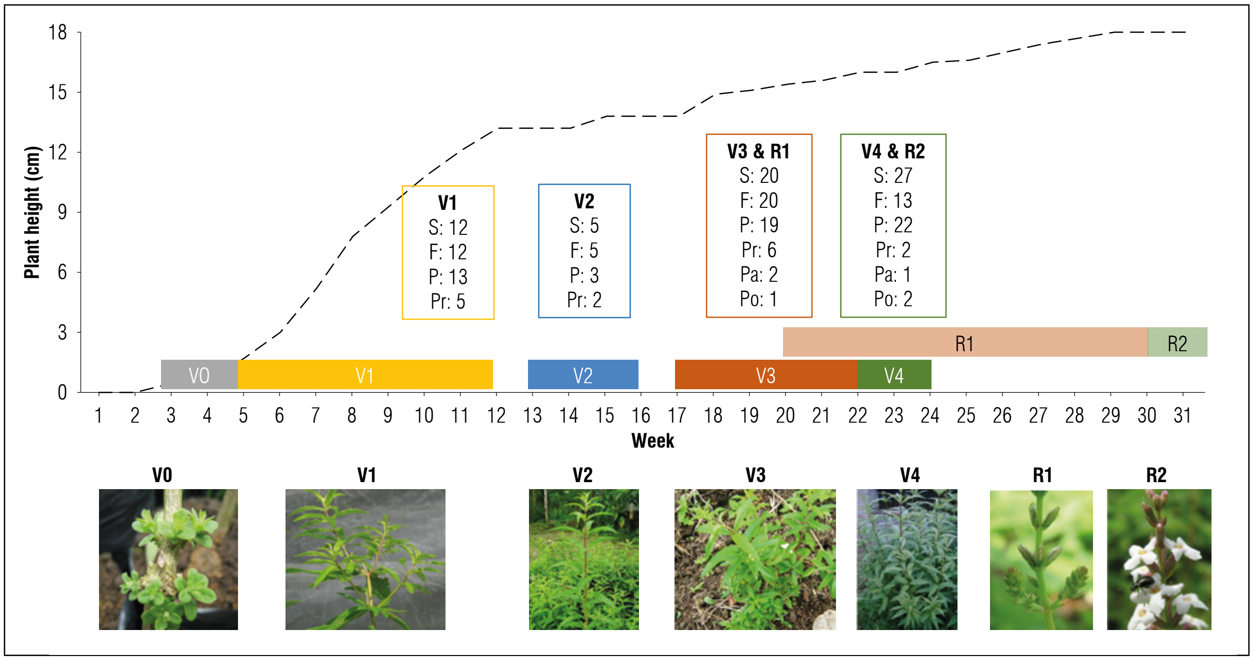

Figure 1. **Relationship of plant phenology, insects, and feeding habits. S: species, F: families, P: phytophagous, Pr: predators, Pa: parasitoid, Po: pollinators.**

of 5 primary branches was maintained, with the appearance of one secondary branch per week. This stage ended with 7 secondary branches, each with an average length of 2.5 cm. Stage V3 began in week 17 and lasted until week 22, ending with plants with 11 secondary branches of 5.5 cm and 3 tertiary branches of 1.2 cm in length. Stage V4 lasted from week 22 to week 24, showing a constant increase in growth and the number of secondary and tertiary branches, accompanied by the elongation of buds in quaternary branches, which on average had 6.8 branches per plant and a length of 1.2 cm (Fig. 1).

Stage R1 corresponded to pre-flowering, which started with the appearance of floral primordia and continued until anthesis; this stage occurred from the 20th week of evaluation and overlapped with stage V3 (Fig. 1). Stage R2 corresponded to flowering, from anthesis to senescence. It took place between weeks 30 and 32 after planting, and the period between flowering and floral senescence was 7 d (Fig. 1).

Although a correlation was not established between climate variables and the vegetative development of the species, the constant growth during the evaluation period, characterized by the increase in the number and length of the branches, could be directly conditioned by abiotic factors such as temperature. Temperature can structurally and anatomically modify plants, favoring the rate of appearance of nodes and leaves when the ranges are optimal for the cultivated species (Pant *et al*., 2021).

Likewise, the temperature in the cultivation area has a direct influence on the essential oil content in different aromatic plants. Specifically in *A. citriodora*, the content of chlorophyll *a*, chlorophyll *b*, carotenoids and flavonoids increases between 5 and 10°C; similarly, at 25°C oxygenated monoterpenes predominate (Rafiee *et al*., 2019). In the study area, where the average temperature is 20°C, this would affect the amount of metabolites, anthocyanins, lycopene, soluble proteins and polyphenols, but would allow for better use of fresh foliage without affecting the growth and productivity of plants due to high temperatures or water stress. However, this topic requires deeper analysis since the development of plants as well as the biosynthesis and storage of secondary metabolites are the result of adaptive processes and survival that involve the interaction between genetics and environmental factors (Pérez-Ochoa *et al*., 2023).

The edaphoclimatic conditions of the area where the species is grown and the type of fertilization have significant effects on the phenology of the plant, even generating delays in the beginning of the reproductive stage. Guzmán-Rivera *et al.* (2004) reported that for the species *Lippia alba* cultivated in Colombia, the plant started the reproductive phase before 50 days after transplanting (DAT), with two peaks defined at 72 and 133 d in the municipality of Candelaria, Valle del Cauca. In Pereira, Risaralda, the reproductive phase occurred at 150 DAT. In the aforementioned areas, stage R2 occurred simultaneously with the vegetative stage, which lasted until flower senescence, similar to what happened with *A. citriodora*.

The variation in time between the vegetative and reproductive stages is important in defining the harvest time because it is directly linked to the amount of essential oil produced and its chemical composition, a determining aspect for the target market. According to Shahhoseini *et al.* (2013), flowering plants have the highest essential oil content. Meanwhile, the harvest of material in the vegetative stage is important for obtaining herbal products, which is how the plant is marketed mainly in Colombia; however, the dry weight of leaves and stems may also vary when the number of cuts increases, affecting the yield of essential oils (Shahhoseini, 2022).

Associated arthropods

The insects associated with lemon verbena plants included 78 individuals belonging to 50 families and 7 orders. Among these were 57 phytophagous, 15 predators, 3 parasitoids, and 3 pollinators. Characterized phytophagous insects include the foliage-eating insects, primarily of the order Coleoptera, family Chrysomelidae, and the genera *Diabrotica*, *Colaspis*, *Epitrix*, *Systena*, *Altica*, and *Cerotoma.* These insects were observed as leaf borers throughout the crop cycle (Fig. 2). The suckers were members of the order Hemiptera, and the mites were members of the group of scrapers.

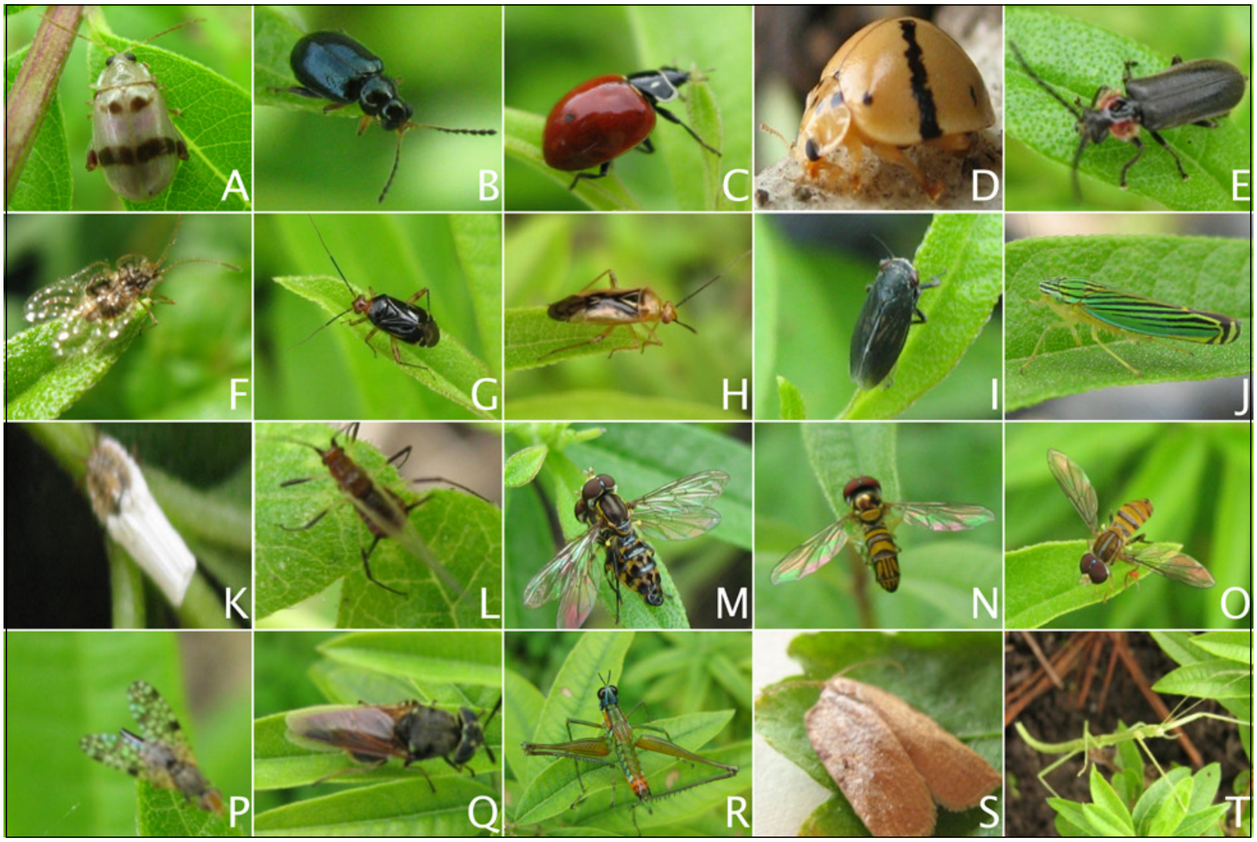

Figure 2. Families of arthropods observed in the crop of *A. citriodora.* A-E, Coleoptera. A-B, Chrysomelidae; C-D, Coccinellidae; E, Cantharidae. F-L, Hemiptera. F, Tingidae; G-H, Miridae; I, Cercopidae; J, Cicadellidae; K, Ortheziidae; L, Aphididae. M-Q, Diptera. M-O, Syrphidae; P, Tephritidae; Q, Stratiomyidae. R, Orthoptera: Eumastacidae. S, Lepidoptera: Gelechioidea. T, Phasmatodea.

The chrysomelids, with chewing mouthparts, causes direct damage to the leaves of plant families, including Verbenaceae, Asteraceae and Solanaceae (Kher *et al*., 2016). These insects are also common to different crops in the Colombian coffee region such as tropical foliage (Aristizabal *et al*., 2013). While their presence is not exclusive to lemon verbena plant, they can be potential pests for the crop, taking into account that they were observed throughout the production cycle and their presence was associated with damage to the foliage.

The insects of the order Hemiptera do not consume the foliage directly, however they do affect the appearance and reduce the vigor of plants by sucking sap from the phloem, causing poor growth, leaf curl, and yellowing and acting as potential virus vectors (although this has not been reported for the species). Mites can cause secondary damage such as chlorosis, color changes and defoliation, reducing the quality of the fresh material (Rehaman *et al*., 2018), and in cases of high infestation, causing the death of the plant.

Direct damage caused by insects restricts the use of fresh foliage and implies an energy expenditure due to the defense process, causing a decrease in the quality of tissues from successive feeding events (Havill and Raffa, 2000), or limiting growth, impeding healthy foliage needed for the production of quality essential oils (Amini *et al*., 2016).

Beneficial arthropod fauna observed included insects that reduce herbivory, among them two types of predators: those with chewing mouthparts, such as the order Coleoptera, family Coccinellidae, genus *Cycloneda* sp. and the order Hymenoptera, family Formicidae, genus *Odonthomachus* sp.; and those with sucking mouthparts such as assassin bugs of the order Hemiptera, family Reduviidae, genus *Zelus* sp., flies of the order Diptera, family Syrphidae, genus *Toxomerus* sp., which also have diverse attack strategies to target organisms (Fig. 2). Members of the family Formicidae establish mutualistic relationships with plants by releasing toxic substances during attack and dissolve the hemolymph of prey in exchange for shelter and food (Arcila-Hernández *et al*., 2017), as well as trophobiosis with members of Hemiptera. Although this interaction was not observed during the study, it is possible that it could occur and that in addition to causing implicit damage, could facilitate the appearance of fungal pathogens due to the secretion of honeydew.

Coccinellids are generalist predators of soft-bodied insects such as aphids and coccids (Aristizabal *et al*., 2013). They are associated with different species such as *Aphis gossypii*, *Myzus persicae*, *Thrips tabaci*, *T. palmi*; *Bemisia tabaci*; *Spodoptera* sp. eggs, mealy bugs (Milán, 2010), Coccidae, Pseudococcidae and juvenile stages of Lepidoptera (Milán *et al*., 2008). Their distribution is related to the availability of prey with preferences depending on the species (Elekcioglu, 2020). Given that the population of aphids observed was greater than that of coccids, those species whose feeding habit is aphidophag are likely favored, representing a potential biocontrol factor in this type of agroecosystems that can eventually be reinforced by flies of the Shyrpidae family that in the larval state are consumers of aphids (Dunn *et al*., 2020) and that were also frequently observed visiting *Aloysia* plants.

In parasitoid insects, specimens of the order Hymenoptera such as the families Braconidae and Tachinidae were found. As with other beneficial insects, their presence is determined by the complexity of the ecosystem and the chemical information they receive from the host plant (Escobar-Escobar *et al*., 2022) and as well as by the simplification of the environment (Tooker *et al*., 2020), such as forest fragmentation, and by the establishment of monocultures. Conversely, the observed presence may indicate a supply of potential hosts, as these species appeared when the plant was in the vegetative stage V4, where the highest number of phytophagous insects was observed (Fig. 1).

Among the pollinators, members of the order Hymenoptera, families Halictidae and Apidae were identified, confirming the qualities of the lemon verbena plant as a melliferous plant, energetically attractive to pollinators and conducive to the appearance of beneficial and generalist organisms such as bees (Botero-Restrepo, 2011). The distribution of the family Halictidae is worldwide, and its members are known as pollinators of more than one hundred crops, including aromatic plants (Rajkumar and Dey, 2016). The presence of Halictidae represents an interesting association, as some species are pollen specialists and have adapted both in behavior and morphology to a specific range of host plants, which in turn are also closely related (Danforth *et al*., 2008).

Likewise, the Apidae family is one of the most ecologically important groups of insects, pollinating wild plants and contributing to the natural balance of ecosystems. According to Arnold *et al.* (2021) its presence is related to the richness of species of trees, plants and herbs with flowers in the interaction zones. This aspect was favored by the accompanying plants on the edges of the crop. Additionally, this reaffirms the beekeeping value of the lemon verbena, with flowers that provide good volume and quality of nectar, essential elements for the sustainability of the species and a potential resource in conservation and pollination programs (Nascimento *et al*., 2014).

Furthermore, the presence of pollinators in an ecosystem is influenced by the direct and indirect services provided by the ecosystem (Arnold *et al*., 2021), such as seed dispersal and pollination (Crespo-Pérez *et al*., 2020), which in turn is related to climatic factors, plant phenology and the type and number of associations established between plants and insects (Cuartas-Hernández and Gómez-Murillo, 2015).

The order Coleoptera was the taxon most frequently observed, with 25% of the total number of specimens identified. Although their role in pollination is not as evident as members of the order Hymenoptera, their abundance in nature suggests that about 90% of pollinated plants have associations with beetles (Wojcik, 2021). Although the size and characteristics of the lemon verbena flowers reduce the possibility that these insects act as pollinators, further research is suggested to determine their participation in this association, which has been considered merely phytophagous.

In general, the presence of beneficial insects does not depend exclusively on agronomic management; other important factors are the supply of food and shelter in plants adjacent to the crop, the structure of the habitat, the complexity of the assemblage in terms of plant species and insect taxa which condition the ecosystem services they can provide (Arnold *et al*., 2021). The plant also has an active role by releasing volatile compounds following herbivory or oviposition, which act as an attractant for parasitoids and predators (Havill and Raffa, 2000; Fürstenberg-Hägg *et al*., 2013). This was observed since most of the beneficial species were associated with vegetative stages where the damage caused by phytophagous was also more evident.

Similarly, the presence and permanence of some predatory insect species in the crop were possibly influenced by the surrounding plants such as Mexican sunflower (*Tithonia diversifolia* Hemsl. A. Gray), crotalaria (*Crotalaria* sp. L.), guandul (*Cajanus cajan* L. Millsp.), and arboloco (*Montanoa quadrangularis* Schultz *Bipontianus*). These plants provided additional ecosystem services, favoring generalist entomofauna. In the specific case of *T. diversifolia*, it serves as a refuge for predatory insects such as wasps, beetles and hoverflies, which were also observed in this context (Baideng *et al*., 2020).

However, a longer period of cultivation is likely required to favor the process of adaptation and conformation of a suitable habitat that supports the increase of the beneficial population of insects, especially those that provide ecological services such as pest regulation. Their increase is favored, among other aspects, by management systems that include cover crops and organic amendments (Adhikari and Menalled, 2020). These practices play a significant role in the interactions between plant and insects by increasing microbial activity in the rhizosphere, changing the nutritional value of plants and defense mechanisms, as well as attracting and maintaining natural enemies (Rowen *et al*., 2019; Adhikari and Menalled, 2020).

CONCLUSION

The study of the phenology of the lemon verbena plants made it possible to identify different stages of plant development, establishing relationships with the associated arthropods and leading to an understanding of the interactions generated between them. This information contributes to the promotion of management plans that promote the increase and establishment of beneficial arthropod fauna. It also helps determine optimal harvesting times, when the species expresses its maximum potential, aligning with market demand.

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