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Ecofungicides as growth stimulants in *Lactuca sativa L.*

Ecofungicidas como estimulantes de crecimiento en *Lactuca sativa L.*

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Abstract

Lactuca sativa commonly known as lettuce is a food that provides few calories due to its high water content and fewer carbohydrates, proteins and fats. The main problem with this crop is that during its cycle it is attacked by a range of fungi (*Fusarium spp., Pythium sp., Alternaria alternata.*, among others) and bacteria (*Erwinia sp, Burkholderia sp*, among others); this leads to excess use of pesticides, economic losses and damage to the environment. Therefore, sustainable control strategies are sought to mitigate the spread of diseases and stimulate the optimal development of the plant. Ecofungicides were made based in: *Ricinus communis* "higuerilla"; *Chamaemelum nobile* "manzanilla" with *Allium sativum* "ajo"; *Ruta graveolens* "ruda"; as well as Bordeaux broth as a craft product and a synthetic product. The randomized complete block experimental design and the Tukey multiple means comparison ($p \leq 0.05$) showed tha in *Ricinus communis* based ecofungicide favors, on average, the height per plant (18.89 cm), the diameter per plant (36.29 cm); the number of leaves per plant (15.50 cm), the leaf width (19.85), the leaf length (17.76 cm) and therefore, a final weight of 1.43 kg per healthy plant was reported. It´s concluded that higuerilla treatment at a dose of 0.33 ml in 1 liter of water increases the productivity of *Lactuca sativa L.* and decreases the incidence of phytopathogenic fungi.

Ecofungicides, Lettuce, *Ricinus communis*

Resumen

Lactuca sativa conocida comúnmente como lechuga es un alimento que aporta pocas calorías por su alto contenido en agua y menor cantidad de hidratos de carbono, proteínas y grasas. El principal problema de este cultivo es que durante su ciclo es atacado por una gama de hongos (*Fusarium spp., Pythium sp., Alternaría alternata*., entre otros) y bacterias (*Erwinia sp, Burkholderia sp,* entre otros); con ello se deriva el exceso de usos de pesticidas, perdidas económicas y daños al medio ambiente. Por lo anterior, se buscan estrategias de control sustentable que permitan mitigar la propagación de enfermedades y estimular el desarrollo óptimo de la planta. Se realizaron ecofungicidas a base de: *Ricinus communis* "higuerilla", *Chamaemelum nobile* "manzanilla" con *Allium sativum* "ajo", *Ruta graveolens* "ruda", así como caldo bordelés como producto artesanal y un producto sintético. El diseño experimental de bloques completos al azar y la comparación de medias múltiples Tukey ($p \leq 0.05$) demostró que el ecofungicida a base in *Ricinus communis* favorece en promedio la altura por planta (18.89 cm), el diámetro por planta (36.29 cm); el número de hojas por planta (15.50 cm), el ancho de hoja (19.85), el largo de hoja (17.76 cm) y por consiguiente, se reportó un peso final de 1.43 kg por planta sana. Se concluye que el tratamiento de higuerilla a una dosis de 0.33 ml en 1 litro de agua incrementa la productividad de *Lactuca sativa L.* y disminuye la incidencia de hongos fitopatógenos

Ecofungicidas, Lechuga, *Ricinus communis*

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Introduction

Lettuce (*Lactuca sativa* L.) is a self-pollinating, dicotyledonous, annual herbaceous plant belonging to the family *Compositae (Asteraceae*), one of the largest and most diverse families of flowering plants. It comprises one tenth of all known angiosperm species. Its Latin name Lactuca is derived from the Latin word "Lac,-tis" meaning "milk", this etymology refers to the milky liquid exuded by the stems of this plant when cut, while the term "sativa" refers to the seed (De Vries, 1997; Simko, 2009).

Mexico is the main supplier of fruit and vegetables to the United States and the value of fruit and vegetable exports has grown at significant rates (Salazar Cantú & Ortiz Gallardo, 2021). In 2020, lettuce production in Mexico was 541,804 tonnes (+5.1% compared to 2019), which were obtained from 22,270 hectares harvested (+7.2%), leaving the national average yield at 24.3 tonnes per hectare. In October 2022, 155. 4 ha sown and a production of 14.81 udm/ha were reported in the state of Hidalgo (SIAP, 2022).

The main problems in lettuce cultivation are related to root diseases caused by phytopathogenic fungi that live in the soil and are difficult to control. These fungi are categorised as opportunistic fungi because of their predisposition to attack crops (Chiles & Andrés, 2018).

Among the main fungal problems that attack lettuce roots are: *Pythium, Fusarium spp., Sclerotinia sp. and Rhizoctonia sp*. among others (Saavedra, Corradini, & Antúnez, 2017). This group of microorganisms enter the plant in association or individually causing the disease known as damping-off or "seedbed rot" which causes chlorotic spots, wilted plants and even the death of the plant (Saavedra, 2017).

The Mexican agrochemicals market was valued at USD 1,268.0 million in 2021 and is estimated to reach USD 1,617.0 million by 2027, registering a CAGR of 4.1% during the forecast period of 2022-2027 (Mordor, 2021).

Furthermore, it should be noted that several studies have reported hormone and endocrine disrupting capacity in human and animal health with the continued use of pesticides (Andrade-Ribeiro, Pacheco-Ferreira, da Cunha, & Mendes-Kling, 2006; Buriticá, 2019; Dutra & Ferreira, 2019; Morales Ovalles, Miranda de Contreras, & Di Bernardo Navas, 2014; Moya *et al*., 2015; Romero Tabares & Tovar Martinez, 2019).

Therefore, this research project proposes to demonstrate the use of ecofungicides to stimulate the growth of lettuce and, in this way, mitigate the use of pesticides and favour the economy of producers.

Theoretical framework

During the growing cycle of Lactuca sativa "lettuce" there are problems caused by: Rhizoctonia solani, *Cylindrocarpo*n destructans, *Fusarium oxysporum*, *Phytopthora sp*, *Phthium sp, Alternaria alternata*; these fungi are controlled by: thiophanate methyl, carbendazim, propiconazole, Mancozeb and Propamocarb hydrochloride.

Phytosanitary problems caused by fungi damage from the seedling and germination process, affecting the roots. It also causes seedling growth to be slow, leading to cankers on shoots, stems and stolons. The rosettes and heads obtained show damage to the leaves (see image 1), which reduces their commercial quality.

Figure 1 Lettuce leaf necrosis
Source: Self Made

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In modern agriculture, chemical control is generally used to combat diseases, as fungicides have become an integral part of efficient food production (Carmona & Sautua, 2017). However, the consequences of overuse have led to the development of ecofungicides.

Ecofungicides are a contribution to sustainable agriculture, and promote social awareness for a better quality of life. Ecofungicides can be defined as "ecological fungicides" because in their development, the aim is to make the final product environmentally friendly and harmless to human and animal health (Li Pun, 2013; Zaker, 2016).

Generally, the frequency of application ranges from 7 to 14 days depending on climatic conditions (Gabriel, Crespo, & Danial, 2013) and several studies report low levels of residual minerals as pure chemical elements such as calcium, sulphur, copper, boron, among others; which do not have prolonged residual powers (Cumara Huaynoca; Gonzales Lugones, 2008; Khan, Ahamad, & Rizvi, 2019).

Therefore, ecofungicides are a viable alternative to achieve agricultural development worldwide.

Methodology

Sowing and transplanting of *Lactuca sativa*

The sowing of romaine lettuce was carried out manually in germination trays using vermiculite as substrate. After 15 days of sowing, the plants were transplanted in an open field under conventional management.

Production of ecofungicides

For the ecofungicide based on *Ricinus communis* "higuerilla", 100 grams of higuerilla leaves were used, which were cut into small pieces and boiled in 1 litre of water for 30 minutes. Once the product had cooled, 2 grams of neutral soap and 3 litres of water were added. An application rate of 50 ml of solution per litre of water was used. In the preparation of the ecofungicide based on *Ruta graveolens* "ruda", 125 grams of fresh leaves were crushed, to which 125 millilitres of hot water were added and left to stand for 24 hours and then the product was strained. Add 2.5 g. of neutral soap and make up to 2.5 litres of water for direct application of the product.

For the ecofungicide based on *Chamaemelum nobile* (chamomile) with *Allium sativum* (garlic), 100 g of fresh chamomile and 100 g of garlic (cut into small pieces) were used. The garlic is boiled in 1 litre of water for 30 minutes, then removed from the heat and the chamomile is added. Leave to stand for 24 hours before applying 1 litre of solution in 5 litres of water.

In the preparation of the Bordeaux broth, 200 grams of copper sulphate were mixed with 200 millilitres of water. In another container, 200 grams of quicklime were diluted in a total of 1800 millilitres of water. Finally, the solutions were mixed by adding the contents of the copper sulphate container to the quicklime container. 5 ml per litre of water was applied.

The synthetic treatment was carried out with the active ingredient oxytetracycline (oxytetracycline hydrochloride) 37.7% + copper (copper oxychloride) 45%, commercially known as Coboxy. At a dose of 2 grams per litre of water.

Consider that all applications were carried out on the same day with a knapsack pump in the morning or in the afternoon directed to the soil.

Statistical analysis

A randomised complete block design with three replications was used. ANOVA was by Tukey significance ($p \leq 0.05$).

Results

Plant height

On October 8, 2022, the height per plant for Bordeaux mixture was 2.29% higher than that of *Ricinus communis*; the latter treatment being the second ecofungicide with the greatest height. Although there is no statistically significant difference ($p \leq 0.05$) as shown in Graph 1, it can be inferred that Bordeaux mixture and Ricinus communis have favoured the metabolic process to stimulate the vegetative cycle of the plant and reflect it in the height. For this date, the control reported the lowest height; in other words, the agronomic management usually carried out by the producer did not have a great impact on this variable.

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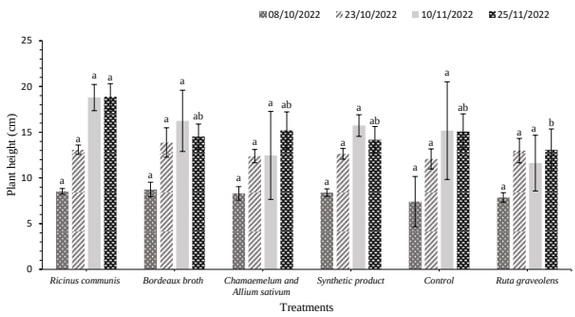
On 23 October 2022, 30 days after transplanting, although there was no significant difference ($p \leq 0.05$) between the treatments, the height per plant for the higuerilla treatment was 5.62% less than the Bordeaux mixture, the latter treatment being numerically better. It should be noted that the control again reported the lowest height.



Graph 1 Comparison of means in height of *Lactuca sativa L* at different dates of application of ecofungicides. Common letter are not significantly different Tukey (*p≤0.05)*

On 10 November 2022, the higuerilla treatment achieved 13.57% more than the Bordeaux broth, this behaviour was contrary to the first dates; however, no statistically significant difference is reported (Tukey *p≤0.05*). Tovar, 2017 reports that the use of higuerilla protects the root, inhibiting the development of phytopathogenic fungi; likewise, it reduces the population and development of insects and larvae, favouring optimal development in the plant. It should be noted that for this date, the treatment that reported the lowest height was the ecofungicide based on rue, leaving the Controllo in fourth place. It is important to mention that during these dates there were extreme temperature changes, which affected the plant and its development.

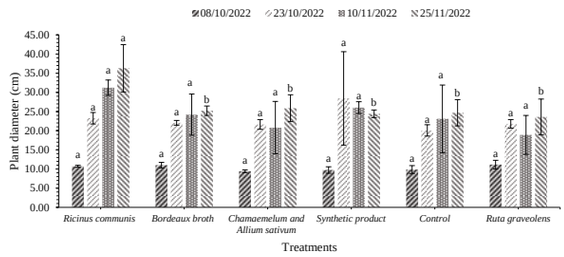
Finally, for 25 November 2022, rue is considered the least recommended treatment for obtaining a favourable height. On the other hand, the higuerilla-based ecofungicide obtained 19.53% more than *Chamaemelum and Allium sativum chamomile* with garlic.

These artisanal products have had an impact on the control of nematodes, fungi and insects. Salinas Tamayo, in 2020 evaluated the antifungal capacity at concentrations of 5 % and 10 % of plant extracts based on chamomile (*Chamaemelum nobile*) and garlic (*Allium sativum*) for the control of *Fusarium oxysporum*.

The results obtained showed that the garlic and chamomile extracts evaluated have an inhibitory effect on mycelial growth and sporulation of *Fusarium oxysporum*. Furthermore, Chávez and Jara, in 2012 evaluated garlic extract to mitigate *Rhizoctonia sp*., *Fusarium sp*. and *Sclerotium sp*. fungi; however, they did not control the disease and concluded that none of the extracts was efficient for the control of Damping off.

Plant diameter

On the first date, the *Ruta graveolens* treatment showed 1.35% more than the Bordeaux mixture (see graph 2). There are antecedents of Gissela in 2020; who evaluated the effect of hydroalcoholic extract based on rue for the management of phytopathogenic fungi in the lettuce crop and reflected the result in the yield with 9222.50 kg/ha. By day 23 October 2022, the higuerilla based ecofungicide recorded, 18.25% less than the synthetic product, although statistically there is no significant difference (*p≤0.05*). According to the 10th of November 2022, the higuerilla treatment achieved 16.62% more than the synthetic product. Even though, statistically there is no significant difference, numerically the handmade product based on higuerilla stood out from all the treatments.



Graph 2 Comparison of means in diameter of *Lactuca sativa* L at different dates of application of ecofungicides. Common letter are not significantly different Tukey (*p≤0.05)*

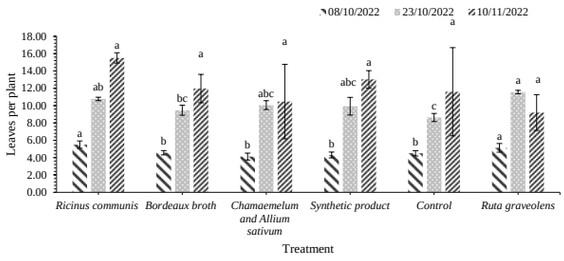
By 25 November, the *Ricinus communis* treatment reported 28.71% more than *Chamaemelum* and *Allium sativum*, reporting a significant difference (Tukey $p \leq 0.05$) between them. The ecofungicide based on higuerilla not only protects the plant from fungi, it was shown to repel insects such as aphids, which were present during the vegetative cycle, the use of this product seeks to consider the integrated management of the crop as established by several authors (Cabra, Rodriguez, & Villota, 2014; Carnero, Medina, Salvatierra, Castillo, & Miranda, 2013; Garzón Espinosa, 2018).

ISSN 2523-6857

The ecofungicide based on *Chamaemelum* and *Allium sativum* proved effective for the control of insects and diseases that usually occur in the crop, this artisanal product is of great advantage due to its low cost and are not harmful to the health of the consumer; in addition, several studies have been reported with the use of these ecofungicides (Crisanto-Pescorán & Aycho, 2013; Chicaré, 2018; Solórzano, Rivadeneira, Alava, & Zambrano, 2019).

Number of leaves per plant

On October 8, 2022, despite reporting a significant difference (Tukey p≤0.05); numerically, the ecofungicide based on *Ricinus communis* obtained 6.73% more than *Ruta graveolens*, with *Ricinus communis* stimulating the growth and development of lettuce leaves on this date.



Graph 3 Comparison of means in number of leaves per plant of *Lactuca sativa L* at different dates of application of ecofungicides. Common letter are not significantly different Tukey (*p≤0.05)*

Graph 3 shows that numerically the *Ruta graveolens* treatment obtained 6.91% more than the *Ricinus communis* treatment on 23 October 2022. Reversing the behaviour of the previous date for both ecofungicides.

By 10 November 2022, the treatments showed no statistical difference (Tukey *p≤0.05*); however, *Ricinus communis* reported 15.94% more than the Bordeaux mixture, the latter product having favourable results reported in agronomy studies (Ramírez-Rodríguez *et al*., 2020).

It is worth mentioning that the use of higuerilla (*Ricinus communis*) has been evaluated in several agricultural variables, mainly for its antagonistic effect (Jena & Gupta, 2012; Rana, Dhamija, Prashar, & Sharma, 2012) and even for phycoremediation of wastewater (Tripathi, Sharma, Purchase, & Chandra, 2021).

On the other hand, the number of leaves per plant was not recorded on 25 November because the crop was at the maturity stage, therefore, it was decided not to count them to avoid damage and economic loss.

The ecofungicide based on *Ruta graveolens* has been used in several research studies mainly for its antioxidant, antimicrobial and antifungal properties. Therefore, it is a favourable option for introducing the plant into the agricultural field (Ari Rojas, 2022; Landi, Peralta-Ruiz, Chaves-López, & Romanazzi, 2021; Szewczyk, Marino, Molinari, Ekiert, & Miceli, 2022).

Leaf width and length

When leaf width was analysed for each of the dates, it was determined that there was no significant difference between treatments; however, leaf width fluctuated from 5.26 cm (*Chamaemelum* and *Allium sativum*) to 5. 55 cm (Control) for the date 08/10/22; from 9.8 cm (Control) to 10.66 cm (*Chamaemelum* and *Allium sativum*) for the date 23/20/22, from 14.4 cm (Control) to 17.26 cm (*Ricinus communis*) and finally from 14.77 cm (*Ruta graveolens*) to 19.85 cm (*Ricinus communis*). With the above, the ecofungicide based on *Ricinus communis* has been efficient for the development of lettuce crop, this product has stimulated height, diameter and number of leaves, promoting optimal growth of the plant. However, there is an area of opportunity to evaluate its role in other leafy vegetables.

According to table 1, on the first date when leaf length was evaluated, no significant difference was reported (Tukey p≤0.05); however, the ecofungicide based on *Ricinus communis* obtained 0.60% more than the Bordeaux mixture. This predicts that these products favour leaf growth.

Treatment	08/10/22	23/10/ 22	10/11/22	25/11/22
Ricinus communis	6.75 (0.43)^{a}	11.34 (0.80)^{a}	15.91 (0.96)^{a}	17.76 (2.14)^{a}
Bordeaux broth	6.71 (0.89)^{a}	11.88 (0.80)^{a}	13.36 (2.51)^{a}	14.30 (1.16)^{ab}
maemelum and Allium sativum	6.45(0.21)^{a}	11.2 (0.67)^{a}	10.92 (3.98)^{a}	14.19 (1.76)^{ab}
Synthetic product	6.58 (0.85)^{a}	10.96 (0.69)^{a}	13.6 (0.80)^{a}	13.77 (1.52)^{ab}
Control	6.66 (0.28)^{a}	10.99 (1.35)^{a}	13 (4.41)^{a}	13.61 (0.88)^{ab}
Ruta graveolens	6.5 (0.52)^{a}	11.64 (1.61)^{a}	9.87 (2.22)^{a}	12.51 (2.32)^{b}

Common letter are not significantly different Tukey (*p≤0.05)*

Table 1 Comparison of means in leaf length of *Lactuca sativa L* at different dates of application of ecofungicides

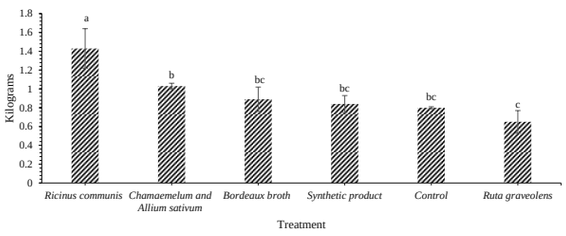
ISSN 2523-6857

According to 23 October 2022, statistically Bordeaux broth obtained 2.03% more than the ecofungicide based on *Ruta graveolens*, however, there is no significant difference (Tukey $p \leq 0.05$) in the treatments. For 10/11/22 there was no significant difference ($p \leq 0.05$) between treatments; however, numerically *Ricinus communis* stands out with respect to the rest of the treatments.

According to the last date of 25 November, a significant difference ($p \leq 0.05$) was reported. However, numerically, *Ricinus communis* obtained 19.49% more leaf length than Bordeaux broth, while statistically it would be the same using Bordeaux broth, *Chamaemelum* and *Allium sativum*, synthetic product and/or the Control (See Table 1). Statistically the ecofungicide based on *Ruta graveolens* was shown to be the least suitable treatment from the previous date.

Final plant weight

According to Graph 4, the ecofungicide based on Ricinus communis favoured the final weight of the product by reporting 54.55% more than Ruta graveolens.



Graph 4 Comparison of means in final weight per plant of *Lactuca sativa L.* Common letter are not significantly different Tukey ($p \leq 0.05$)

Graph 4 shows a significant difference (Tukey $p \leq 0.05$) in the final weight of the treatments. The ecofungicide based on Ricinus communis proved to be favourable for the lettuce crop followed by *Chamaemelum* and *Allium sativum.* Statistically, the Bordeaux mixture, the control and the synthetic product were the same. However, the least recommended ecofungicide is *Ruta graveolens* because it showed less effectiveness in the variables.

It is important to consider and highlight the frequent use of *Ricinus communis* to control and mitigate pests and diseases in crops, however, it is suggested to carry out a precise study on the effects and adverse effects that this product could cause in plants and in the health of consumers.

Conclusions

This ecofungicide based on Ricinus communis is economically profitable for the farmer, because the plant adapts to any place; in addition, it provides development and growth to the plant which is reflected in the size, volume, vigour and bright green colour; being liked by the farmers because with less fertilisers a profitable production was achieved without saturating the plant with synthetic products.

The scientific community is invited to test and evaluate Ricinus communis on growth and adverse effects on other types and species of vegetables.

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