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Agroecological Management of *Sphenarium Purpurascens* with Vegetable Extracts and Entomopathogenic Fungi in Amaranth, Puebla-Mexico

Emmanuel Rodríguez-Palma
Centro de Agroecología del Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla. Edificio Val 1. Km 1.7 carretera a San Baltazar Tetela, San Pedro Zacachimalpa. CP 72960, Puebla, Pue., México

Agustín Aragón-García (Corresponding Author)
Centro de Agroecología del Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla. Edificio Val 1. Km 1.7 carretera a San Baltazar Tetela, San Pedro Zacachimalpa. CP 72960, Puebla, Pue., México
Email: miguel.aragons@correo.buap.mx

Betzabeth Cecilia Pérez-Torres
Centro de Agroecología del Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla. Edificio Val 1. Km 1.7 carretera a San Baltazar Tetela, San Pedro Zacachimalpa. CP 72960, Puebla, Pue., México

Jesús Francisco López-Olguín
Centro de Agroecología del Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla. Edificio Val 1. Km 1.7 carretera a San Baltazar Tetela, San Pedro Zacachimalpa. CP 72960, Puebla, Pue., México

Omar Romero-Arenas
Centro de Agroecología del Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla. Edificio Val 1. Km 1.7 carretera a San Baltazar Tetela, San Pedro Zacachimalpa. CP 72960, Puebla, Pue., México

Miguel Aragón Sánchez
Centro de Agroecología del Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla. Edificio Val 1. Km 1.7 carretera a San Baltazar Tetela, San Pedro Zacachimalpa. CP 72960, Puebla, Pue., México

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Abstract

Among the most important pests of amaranth (*Amaranthus hypochondriacus* L.) is *Sphenarium purpurascens* Charpentier, 1845 (Orthoptera: Pyrgomorphidae) which causes damage to the plant, decreased performance and economic losses, with chemical products being the main control method. The objective of this work was to evaluate the effect of aqueous extracts of *Ricinus communis* and *Capsicum frutensis* combined with two entomopathogenic fungi; one commercial and one native to Puebla-Mexico, alternated with the application of soap, in laboratory and field conditions. Bioassays were carried out to evaluate the compatibility of aqueous extracts with the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* at concentrations of 25, 50 and 75 % in conidia, in addition, six treatments with five repetitions were tested in a randomized block experimental design in the field. Los bioensayos de laboratorio presentaron diferencias significativas en el desarrollo micelial y tasa de crecimiento, donde se observó un estímulo positivo en el crecimiento de *M. anisopliae* por parte de los extractos vegetales. In the field, the H+Ch+M treatment registered the least damage (12.9%), least infestation (1.1%) and best seed production (1,353.7 kg/Ha). The effect of aqueous extracts combined with *M. anisopliae*, alternated with soap applications, proved to be an effective strategy in the integrated management of S*phenarium purpurascens*, in amaranth cultivation in Puebla, Mexico.

Keywords: Yield; Chapulin; Chicalote; Higuerilla.

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1. Introduction

The Orthoptera order has come to take on great agricultural importance in Mexico, because it has become a pest that causes economic losses between 20 and 30 % of production when control actions are not carried out Vázquez, *et al.* [1]. Within this order is the species *Sphenarium purpurascens* Charpentier, 1845 (Orthoptera: Pyrgomorphidae), this species in Mexico has caused great agricultural losses in crops of amaranth, beans, corn, pastures and some fruit trees mainly being the most affected Aguascalientes, Coahuila, Chihuahua, Durango, State of Mexico, Guanajuato, Hidalgo, Michoacán, San Luis Potosí, Tlaxcala, Veracruz, Zacatecas and Puebla [2, 3]. The most common methods for its control are based on the use of chemical insecticides, such as parathion and malathion that belong to the Organophosphate family, and carbaryl within the Carbamate family, these products have caused damage to the environment, and generate resistance in populations of pest insects [4, 5].

Among the innovations that are available to counteract the damage caused by insect pests without having to apply chemical products, is the use of wild plants as biorepellents; natural insecticides based on plant extracts

constitute an interesting alternative for insect control [6]. With this approach, Pérez-Torres, *et al.* [7] report that there are some plants that have been used to control pests in the cultivation of amaranth, *Ricinus communis* L. 1753 known as castor, *Cratón ciliatoglanduliferus* Ortega, known in the state of Puebla as soliman, both species of the family Euphorbiaceae; *Argemone mexicana* L. (Papaveraceae) commonly called chicalote, *Psacaliopsis purpusii* (Greenm. ex Brandegee) H. Rob & Brettell (Asteraceae) known as doggrass and *Capsicum frutescens* L. (Solanaceae) known as chili, the selection of this type of plants is very important, since botanical biorepellents can be prepared from these.

The fruit of *C. frutescens* contains a group of capsaicinoid compounds, among which are capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin, whose insecticidal properties have been reported as an odorless alkaloid, which has been tested against flies *Bemisia tobaci* (Gennadius, 1889) (Hemiptera: Aleyrodidade), the aphid of the genus *Macrosiphum*, the grasshopper, the coffee borer *Plagiohammus colombiensis* (Coleoptera: Cerambicidae), among others [8].

Ramos, *et al.* [9], reported that castor oil and ricinin are the active ingredients of *R. communis*, which acts against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), in addition to showing better insecticidal activity with seed extracts than with leaf extracts. Salinas [10], cited that all parts of the *R. communis* plant are toxic, especially the seeds that present two important substances: a) Ricin, with a highly toxic protein structure (toxoalbumin) and a crystalline nitrogenous body that has some characteristics of alkaloids and b) triglycerides of rhinoleic acid.

Another widely used tool for pest control is the use of entomopathogenic fungi, these fungi are a group of microorganisms with more than 700 species within 90 genera that can infect insects [11, 12]. Diseases caused by insect fungi significantly reduce populations, thus demonstrating that bioinsecticides can be a viable alternative to solve pest problems in agriculture [13, 14]. Among the fungi most used as biological insecticides are *Metarhizium anisopliae* and *Beauveria bassiana*, where *M. anisopliae* attacks more than 200 species of insects and mites of different genera, in the orders Orthoptera, Hemiptera, Lepidoptera, Dermaptera, Hymenoptera, Coleoptera, among others. others [15, 16]. In addition, *B. bassiana* infects a wide range of hosts, which is why it is used as a biopesticide against different genera of insects, where it has been tested to control *S. purpurascens* by penetrating the insect cuticle and has good results in its use management [17].

The use of various tools within Integrated Pest Management guarantees adequate control of pest populations, as long as these tools are complementary and the effect they present does not counteract each other. Therefore, the objective of this research was to evaluate the effect of combining the aqueous extracts of *R. communis* with *C. frutescens* and the entomopathogenic fungi *B. bassiana* (commercial strain B-BLAS) and *M. anisopliae* alternated with the application of neutral soap, as a strategy in the agroecological management of *S. purpurascens* in the cultivation of amaranth in Puebla, Mexico.

2. Materials and Methods.

Both the preparation of the plant extracts and the laboratory tests with the plant extracts and entomopathogenic fungi were carried out at the Genetic Resources Center of the Centro de Agroecología of the Universidad Autónoma de Puebla.

2.1. Preparation of Plant Extracts

For the preparation of plant extracts based on *R. communis* and *C. frutescens*, the methodology proposed by Aragón, *et al.* [18], which consists of collecting the ripe fruit of *R. communis* from the plant that grows sporadically, letting it dry in the shade for 20 days until dehydration, once the plant material is dry, it is pulverized with an electric grinder (Nixtamatic), this powder It was stored in paper bags and left in a cool place until use. for the preparation of the *C. frutescens* extract, 24 hours before use, the fresh fruit of the plants grown in the region was collected under standard cultivation conditions and blended in a blender (Osteriser). For both extracts (*R. communis* and *C. frutescens*) they were poured into water for 24 hours before application to extract water-soluble compounds at a dose of 3% (30 g of plant material per liter of water), subsequently, it was adjusted to a concentration of 50% of the aqueous extract for its application in the different treatments, prior to the application it was filtered with a fine mesh to separate the solids from the liquids.

2.2. Bioassays in Laboratory

The strain used in the present investigation is the CP-MA1 of *M. anisopliae*, which was collected in cultivation plots belonging to the Municipality of Tetela de Ocampo, Puebla-Mexico, this strain was isolated and has been maintained on potato dextrose agar (PDA) medium at the Genetic Resources Center of the Centro de Agroecología of the Instituto de Cienciasof the Benemérita Universidad Autónoma de Puebla.

To evaluate the compatibility of the plant extracts with *M. anisopliae*, four treatments with five replicates were tested in a completely randomized design: 1) PDA + *C. frutescens* (PDA+Ch), 2) PDA + *R. communis* (PDA +H), 3) PDA + *C. frutescens* + *R. communis* (PDA+H+Ch), 4) PDA (control).

For the preparation of the culture media, 7.8 g of PDA was diluted in 200 mL of distilled water and the aqueous extracts of *C. frutescens* and *R. communis* were mixed at different doses, using 7.5 g of extract for a concentration of 25 %, 15 g for a concentration of 50 % and 22.5 g for a concentration of 75 % of each one, it was shaken and sterilized at 120 °C for 15 min, it was allowed to settle and then poured into sterile Petri dishes (4.5 cm diameter), while for the elaboration of the control treatment the supplier's methodology was followed. With a 0.5 mm sterile

bag, PDA cuts containing *M. anisopliae* mycelium were made and deposited on the inside edge of each Petri dish, closed and sealed with parafilm, this procedure was carried out for each of the treatments and for the three concentrations with their repetitions.

The macroscopic characteristics of the CP-MA1 strain of *M. anisopliae* were recorded at a temperature of 27 ± 2 °C, relative humidity 80 ± 5 % and photoperiod at 12:12 light-dark for 15 days, which were: density, air mycelium and color. The growth rate and development rate were determined every 24 hrs using the following formula proposed by Rojas and Hormaza [19]:

$$TD = \frac{CF - CI}{TF}$$

Where:
CF: Final growth.
CI: Initial growth.
TF: Final time.

Data collection was stopped when the growth of the fungus in the control treatment covered 100% of the PDA surface inside the Petri dish.

2.3. Field trials

The field work was carried out in a demonstration plot planted with amaranth Amaranthus hypochondiacus L. (Amaranthaceae) belonging to the community of San Lucas Tulcingo in the municipality of Tochimilco, Puebla-Mexico. This locality presents an altitude of 1,950 masl, whose geographical coordinates are 18º 50 '14' 'of north latitude and 98º 35' 42 "of western length. In the region four types of climate predominate: a) subhumid climates with summer rains in the lowest area of municipality, b) subhumid temperate climate with summer rains that occur in the lower slopes of the Sierra Nevada, c) semi-deciduous climate sub-humid located in the intermediate zone between the lower slopes of the mountain range and the highest part of the volcano Popocatepetl and d) cold climate located in the highest part of the volcano, the predominant type of soil is Andosol [20].

The experimental plot of 2000 m^2 had 1,800 plants, 60 plants in the experimental unit and 12 plants in the useful plot, each plant was made up of three amaranth plants. The experimental design was randomized complete blocks where six treatments with five repetitions were tested. For the study, a strain of *B. bassiana* commercially available in the city of Puebla-Mexico was taken as a reference in the company "Agrobionsa de México", where the B-BLAS product was purchased at a concentration of 1x108 spores/mL and a viability of 94%. In the case of the CP-MA1 strain of *M. anisopliae*, it was reactivated in Sabouraud-Dextrose-Agar (SDA) medium plus 0.5 mg L-1 of Chloramphenicol at 27 ± 1 °C. Subsequently, it was reproduced massively in 300 g of sterilized rice in polypaper bags, for which 5 previously colonized Petri dishes were placed 5 mL of sterile water and with the help of a bacteriological handle it was mixed with the spores, later 5 mL suspension was inoculated. of conidia to sterilized rice under aseptic conditions in an isolation chamber and incubated for 20 days at 28°C. To obtain the spores, 500 mL of sterile distilled water were added, gently rubbing the rice, the concentration of conidia was determined in a proportion of 1/10 by counting in a Neubauer chamber, adjusting the suspension to 1x108 spores/mL, the suspension obtained was kept refrigerated until use [21]. For the evaluation of the germination of conidia, the methodology of Marín and Bustillo [22] was followed, five points were placed with a suspension of 1x108 spores/mL in Petri dishes (100 x 15 mm) with PDA. 100 spores were observed and the number of germinated spores from the five points was recorded and this value directly represented the germination rate as a percentage of an experimental unit, resulting in a field application of 89%.

The treatments that were established were in the demonstration plot: 1) R. communis + C. frutescens (H+Ch), 2) B. bassiana®, 3) M. anisopliae, 4) R. communis + C. frutescens + B. bassiana (H +Ch+B®), 5) R. communis + C frutescens + M. anisopliae (H+Ch+ M), 6) Water (Control), table 1.

The treatments were applied in the morning with a manual sprinkler between 7 and 10 hrs. The applications began when the plant had an average height of 10 cm and still did not show damage by *S. purpurascens*, these applications were alternated with the application of neutral soap; For this application, 100 g of Zote® soap bar were used in 15 L of water, letting it rest for 24 hours to have a better dissolution, it was filtered and applied. Each treatment was applied once a week, making a total of eight applications (one week the plant extract was applied and then the soap). The entomopathogenic fungi were applied in suspension at 1x108 spores/mL (two grams of spore powder per liter of water), which were applied with the first application of the vegetative extract and one month later. For the control treatment, only purified water was applied every week on eight occasions.

The data recorded during the development of the experiment in the field were: a) number of *S. purpurascens* present in each of the plants of the useful plot; b) damage of the amaranth foliage in the useful surface; based on the percentage of the affected leaf area presented by the plants, using a visual scale and taking the total of the plant as 100% according to the methodology proposed by Vázquez, *et al.* [1], and c) total production, when the plants reached physiological maturity, cut the panicles of the plants of the useful plot and dried in the sun for 30 days, threshed, cleaned and weighed the amaranth grain.

For the comparison of means, the F test of analysis of variance (ANOVA) was performed followed by the Tukey test (α = 0.05) to determine if there was a significant difference between the treatments, considering the robustness with respect to the hypotheses of normality and of homoscedasticity. Analyzes were performed with the STATGRAPHICS Centurion XVI.I program.

3. Results and Discussion

3.1. Bioassays in Laboratory

Figure 1 shows the analysis of the development rate of *M. anisopliae*, referring to its compatibility under the effect of plant extracts based on castor (*R. communis*), chili (*C. frutescens*) and their combination, at a concentration of 25, 50 and 75%, where it is observed that in the lower concentration (25%) there are no significant differences between the effect of the treatments, however in the concentration of 50% of the plant extracts there are significant differences ($F_{3, 16} = 5.9$, $P < 0.006$), in the highest concentration (75%) of the plant extracts, it was observed that the rate of development decreases, these results agree with those reported by Castiglioni, *et al.* [23], who report that the neem-based plant extract (Nimkol-L®) at low concentrations positively affected the germination of conidia of *M. anisopliae* strain 1037 and *B. bassiana* strain 634, however, they report that at higher concentrations (5%), the germination of conidia in both strains is affected. These results also agree with Barajas, *et al.* [13] who tested the growth rate of the Bb001 strains of *B. bassiana* and Ma002 of *M. anisopliae* at different temperatures, concluding that the growth, sporulation and germination of conidia is optimal at temperatures between 25-30 °C. Plant-based bioinsecticides have the advantage of being compatible with other management options for insect control, such as pheromones, oils, soaps, predators, parasitoids, and entomopathogenic fungi, as observed in the present investigation, which increases their chances of integration into a pest control and environmentally friendly program [1].

Figure 2 shows the growth of *M. anisopliae* under the effect of the two extracts evaluated and the combination of them, with respect to the growth rate on *R. communis*, a greater growth of the fungus is observed during the first day, this may be due to the fact that the secondary metabolites activate the growth process of the fungus in the first hours of sowing, which allows growth exponentially [24]. For the extract of *C. frutescens* it registered a slower growth in the first days, however on the fourth day it surpassed the extract of *R. communis*, a similar behavior can be observed in the combined treatment of the plant extracts, while the control treatment its growth remained below the other treatments, being statistically different from the other treatments.

The analysis of the development rate of the fungus *M. anisopliae*, in terms of its compatibility under the effect of the different treatments at a concentration of 50% is observed in figure 3, the means were significantly different ($F_{3, 16} = 7.7$, $P < 0.002$), where we observed that there are three groups of means, finding that the PDA+H+Ch treatment was the one that presented the highest growth rate (0.20 mm), followed by of PDA+Ch and PDA+H treatment, while the control was the one with the lowest growth with 0.06 mm. This same behavior was observed growth speed in a concentration of 75% (Figure 4), where the best treatment was PDA+H+Ch, it should be noted that the treatments had an increase in days in terms of growth speed with respect to the other concentrations tested. These results agree with those reported by Castiglioni, *et al.* [23], who found positive viability between the entomopathogenic fungi *M. anisopliae* and *B. bassiana* with aqueous extracts of *Azadirachta indica*, demonstrating that plant extracts do not inhibit their growth in *M. anisopliae* al. 1% and *B. bassiana* with 1.8%. Studies on the feasibility of using the combination of entomopathogenic fungi with plant extracts are very scarce; With these results, it can be demonstrated that the presence of capsaicin and ricin (highly toxic) have insecticidal activity, and do not inhibit the growth of the CP-MA1 strain of *M. anisopliae*, and it is expected that they enhance the effect of both control strategies. of *S. purpurascens* [8, 10]. On the other hand, Hernández-Rosas, *et al.* [12] They mention that *M. anisopliae* has a high degree of germination when combined with multiple essential oils, mainly oils based on garlic, cypress, eucalyptus, lemon and orange, which presented a germination of more than 90 %, making them compatible in many cases the tool of entomopathogenic fungi and plant extracts.

3.2. Field Essay

Regarding the percentage of damage by *S. purpurascens* in the amaranth crop, from 26 days after sowing, there was a significant difference between the treatments, being the treatment based on *R. communis* + *C. frutescens* + *M. anisopliae* who registered less damage compared to the other treatments with an approximate average of 12% damage. This may be because the combined effect of the plant extracts with their insecticidal effects and the effect of the enotomopathogenic fungus *M. anisopliae*, are an effective combination for the control of *S. purpurascens* in amaranth plants. The treatment with the highest percentage of damage was the control group (approximately 19 % damage), presenting significant differences with the other treatments (table 1). This statistical difference between the treatments is maintained as the experiment progressed, reaching day 82 of the experiment, where a clear difference can be observed in terms of the percentage of damage in the control treatment (approximately 40%) compared to the other treatments. These results agree with what was reported by Vázquez, *et al.* [1] where they mention that the application of castor and chili extracts combined with the *B. bassiana* fungus present a percentage of damage of less than 15 % in a period of 50 days for pests that attack the foliage of the amaranth crop, results similar to those obtained in this investigation where a percentage lower than 15 % was obtained in all the treatments for the control of the grasshopper. It should be noted that the combination of entomopathogenic fungi with plant extracts has been tested in other experiments and on other pests, where it has been shown that they have a high potential to combat pests, Cerna-Chávez, *et al.* [25] indicate an increase in the control of several *M. anisopliae* strains when combined with seed extracts of jicama *Pachyrhizus erosu* and *Piper nigrum* L. pepper for the control of *Conotrachelus dimidiatus* (Coleoptera: Curculionidae).

The data obtained in the evaluations of the number of grasshoppers per useful plot are observed in table 2, the presence of *S. purpurascens* can be observed from the first beginnings of the experiment, having a homogeneous distribution in the plot, since the treatments do not present statistically significant difference, with an average of 3

grasshoppers in a useful plot, these values are maintained until day 61, when a growth can be observed in terms of the number of organisms in the control treatment compared to the rest of the treatments (with an average of 5 grasshoppers in a useful plot), after 82 days of the experiment, it is observed that the control treatment was the one that presented the highest number of grasshoppers (with an average of 4 per useful plot) compared to the rest of the treatments, where the treatment of *R. communis* + *C. frutescens* + *M .anisopliae* was the one that presented the lowest number of organisms per useful plot (approximately 1 organism). These data are consistent with those reported by Vázquez, *et al.* [1] where they reported that amaranth plants treated with plant extracts of *R. communis* and *A. mexicana* and the application of *B. bassiana* presented the lowest infestation by *S. purpurascens* during the entire amaranth cycle in the State of Puebla, considering that these authors report a greater number of grasshoppers in the plants.

The production of amaranth per useful plot is observed in table 3, where an increase in the production of all the treatments with respect to the control is shown, which indicates that these control methods are efficient for the control of pests in the cultivation of amaranth. The treatment with *R. communis* + *C. frutescens* + *M. anisopliae* had the best average yield with 1,353.7 kg/ha of amaranth seed with an increase in production of 149% compared to the control treatment, the control treatment presented the lowest production with 543.2 kg/ha. Results similar to those reported by Vázquez, *et al.* [1], where they mention an increase in the production of the amaranth crop of more than 90 % compared to the control in treatments where some plant extract has been applied in combination with the *B. bassiana* fungus. Similarly, these data agree with Pérez-Torres, *et al.* [7] who reported that, by fighting the pests of the amaranth crop with plant extracts, alternating with the application of bar soap, in the Tehuacán Valley, Puebla, the treatment protected against damage caused by foliage insects, which caused an increase in seed production. 78% greater than control. The control of *S. purpurascens* when applying *R. communis* + *C. frutescens* + *M. anisopliae* may be due to the decrease in the number of population present in the crop, due to the effect of capsaicin whose insecticidal properties have been reported as an odorless alkaloid and with lipophilic properties Reyes, *et al.* [8], it may also be that the toxic effect reported by ricin, present in *R. communis* [10] where it influences the number of organisms and if we consider that one of the effects of soap is the decomposition and destruction of the cuticle and membranes. cellular [26], so they are more sensitive to entomopathogenic fungi; They have the ability to synthesize toxins that are used in the pathogen-host relationship cycle. In addition, the form of action is to adhere to the integument and germination of conidia or spores, then penetration occurs through the cuticle of the insect, multiplication of the fungus in the hemocoel and production of toxins and that is when the death of the host occurs in a period of 48 to 60 hours [27].

4. Conclusions

Plant extracts at concentrations of 25 and 50% based on *R. communis* and *C. frutescens*, as well as their combination, positively stimulated the growth and rate of development of CP-MA1 of *M. anisopliae*.

In the case of field bioassays, the treatment based on *R. communis* + *C. frutescens* + *M. anisopliae*, alternated with soap applications, showed the highest efficiency to control the population of *S. purpurascens* in amaranth cultivation, as well Likewise, this same treatment increased the production of this crop by 149%, so this alternative proves to be a strategy that can be used for Integrated Pest Management.

Table-1. Mean ± standard error of the damage percentage of the amaranth plant (*A. hypochondriacus* L) for each treatment after sowing in Tochimilco, Puebla-Mexico

Treatment	Composition	% of amaranth damage (*A. hypochondriacus* L)						
		Days						
		26	33	47	54	61	75	82
Control	Water	19.5±0.7 a	22.1±2.4 a	23.8±2.0 a	26.5±0.7 a	33.6±1.4 a	36.2±0.2 a	40.1±2.5 a
H+Ch+B®	Higuerilla+Chile+B®	17.7±1.2 ab	14.5±1.7 b	15.5±0.4 b	11.1±0.4 b	12.9±1.9 b	13.0±1.1 b	9.8±0.9 b
H+Ch	Higuerilla+Chile	14.4±1.0 ab	16.2±0.8 b	13.7±0.7 b	11.09±0.5 b	10.9±1.4 b	14.1±0.8 b	9.3±0.6 b
B®	B. bassiana	14.5±1.2 ab	13.2±1.4 b	13.9±0.3 b	11.5±0.4 b	14.6±1.3 b	11.6±0.5 b	9.9±0.5 c
M	*M. anisopliae*	15.4±1.2 bc	14.6±1.7 b	14.2±0.5 b	16.4±0.6 b	12.08±1.6 b	12.1±1.1 b	8.4±0.4 b
H+Ch+M	Higuerilla+chile+M	12.9±0.5 c	14.0±1.7 b	14.1±0.4 b	13.4±0.2 b	13.4±0.6 b	12.9±1.5 b	9.9±1.1 c

***** Means with the same letter are not significantly different (Tukey, P <0.05).

Table-2. Mean ± standard error of the number of *S. purpurascens* in the amaranth plant (*A. hypochondriacus* L) for each treatment after sowing in Tochimilco, Puebla-Mexico

Treatment	Composition	Number of *S. purpurascens* in amaranth (*A. hypochondriacus* L)						
		Days						
		26	33	47	54	61	75	82
Control	Water	3.1±0.1a	1.7±0.2a	1.9±0.4a	2.4±0.7a	5.1±0.3a	3.9±0.1a	4.6±0.2a
H+Ch+B®	Higuerilla+Chile+B®	3.1±0.2a	1.6±0.2a	2.3±0.4a	1.3±0.4a	2.6±0.3b	2.6±0.1b	2.5±0.2b
H+Ch	Higuerilla+Chile	3.2±0.2a	1.8±0.4a	3.0±0.7a	1.7±0.5a	2.8±0.3b	2.5±0.1b	2.3±0.2b
B®	B. bassiana	3.1±0.3a	1.8±0.3a	2.1±0.3a	1.5±0.4a	3.2±0.3b	2.5±0.1b	2.8±0.2b
M	*M. anisopliae*	3.1±0.2a	1.6±0.3a	2.0±0.5a	1.9±0.6a	2.9±0.3b	2.5±0.1b	2.7±0.2b
H+Ch+M	Higuerilla+chile+M	3.2±0.3a	1.7±0.1a	2.3±0.4a	1.9±0.2a	0.8±0.3c	0.7±0.1c	1.1±0.2c

* Means with the same letter are not significantly different (Tukey, P <0.05).

Table-3. Mean ± standard error and homogeneous groups of amaranth production (*A. hypochondriacus* L) for each treatment after sowing in Tochimilco, Puebla-Mexico

Treatment	Composition	Mean ± standard error	Increase in production
		Dry weight (kg/ha)	(%)
Control	Water	543.2±22.69 a	-
H+Ch+B®	Higuerilla+Chile+B®	1033.0±71.93 b	90
H+Ch	Higuerilla+Chile	1038.7±119.89 b	91
B®	B. bassiana	1044.0±58.32 b	92
M	*M. anisopliae*	1095.2±22.08 bc	101
H+Ch+M	Higuerilla+chile+M	1353.7±21.36 c	149

* Means with the same letter are not significantly different (Tukey, P <0.05).

Figure-1. Developmental rate of CP-MA1 of *M. anisopliae*, under the effect of plant extracts and their combination at a concentration of 25, 50 and 75%. Averages with the same letter do not present significant differences between them (Tukey, P <0.05)

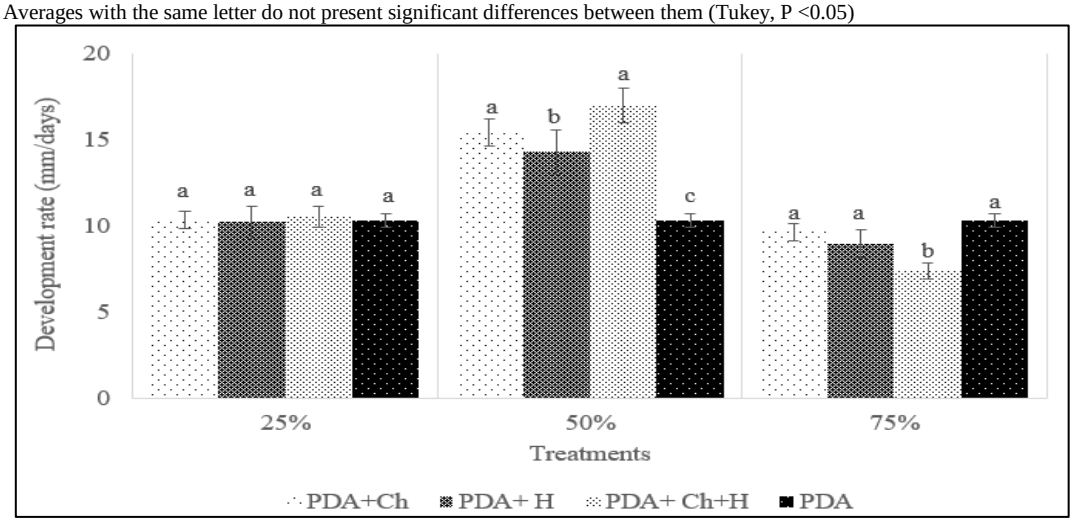


Figure-2. Growth rate of the CP-MA1 of M. anisopliae, under the effect of plant extracts and their combination at a concentration of 25%

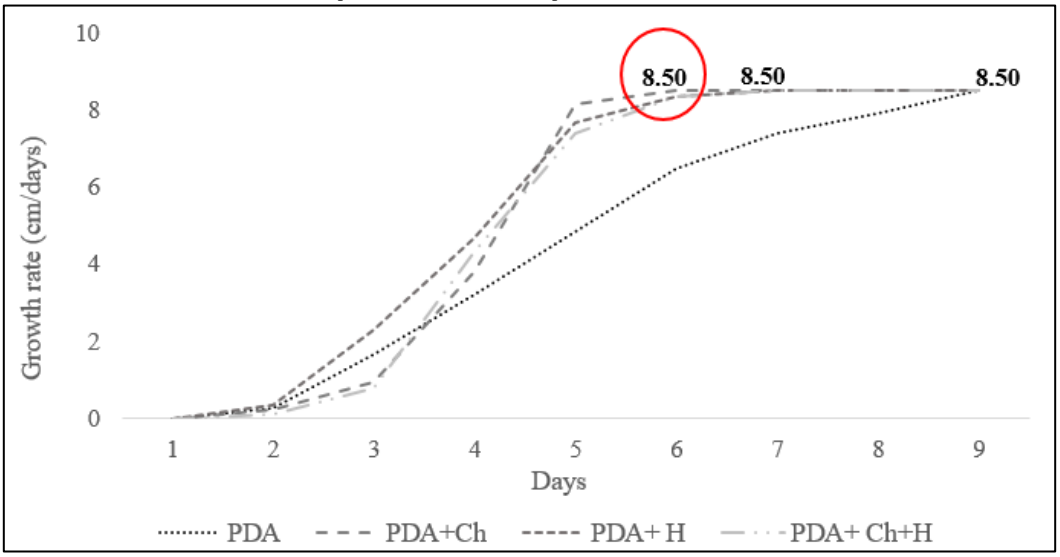


Figure 3. Growth rate of the CP-MA1 of *M. anisopliae*, under the effect of plant extracts and their combination at a concentration of 75%

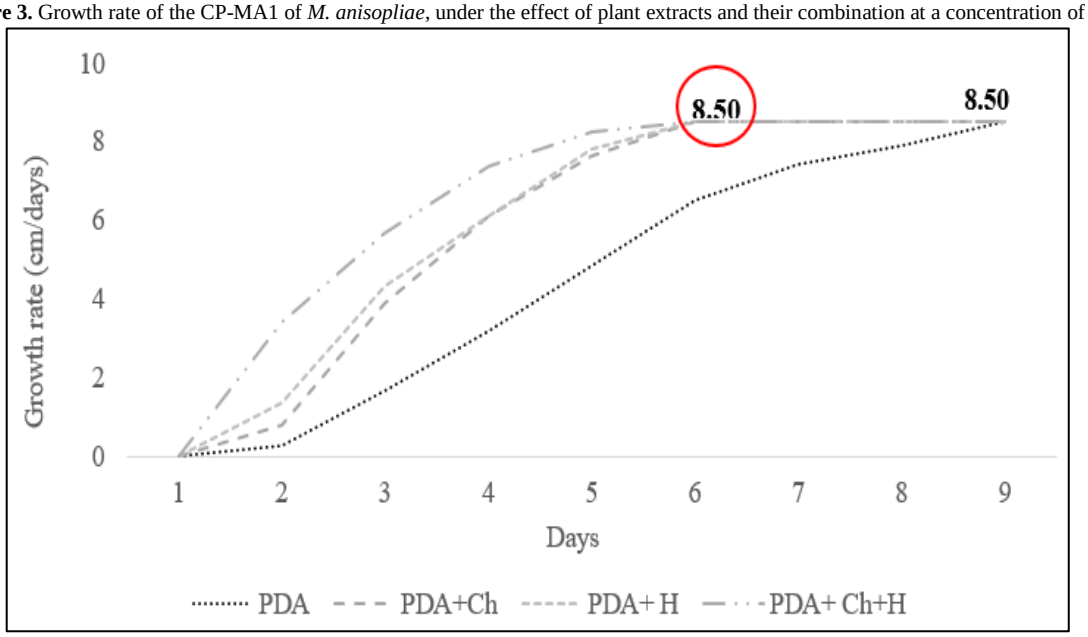
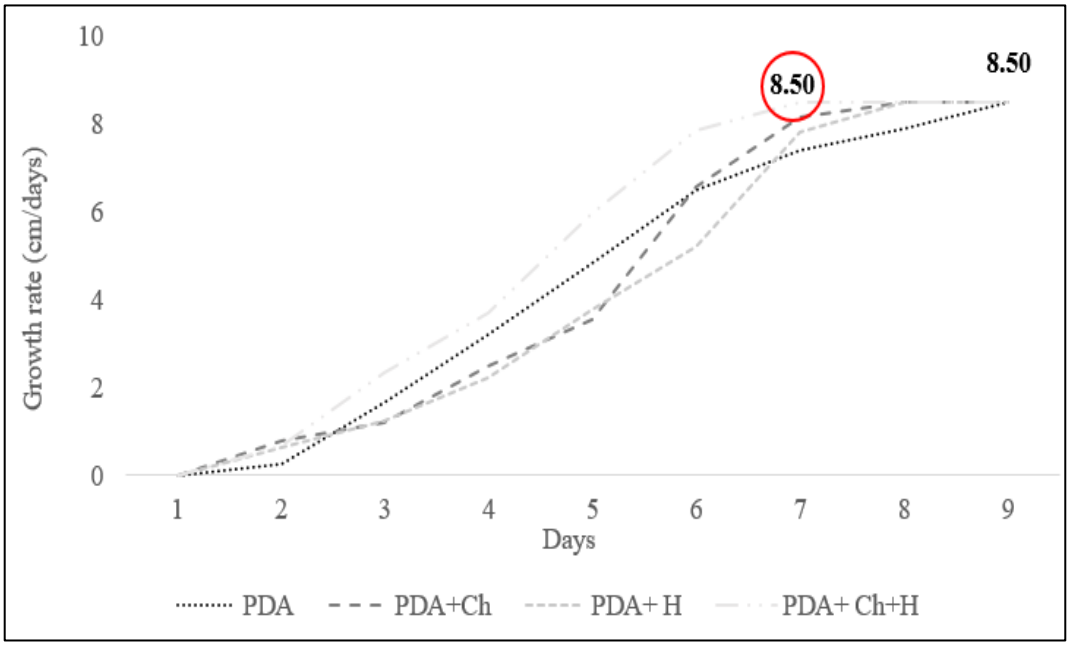


Figura-4. Growth rate of CP-MA1 of *M. anisopliae*, under the effect of plant extracts and their combination at a concentration of 75%



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