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Article

Caracterización y control de la caída foliar en pinos de Puebla

Characterization and control of leaf shedding in pines of *Puebla*

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Resumen

A partir de 2014, diferentes especies de pinos de la Sierra Norte de Puebla han presentado defoliaciones importantes. En el presente estudio se caracterizaron los agentes causales y se evaluó la efectividad de productos químicos y biológicos para el control de la caída foliar en dichos taxones. Se establecieron tres sitios experimentales en los municipios Tetela de Ocampo (1) y Zautla (2). Se siguió un diseño experimental de bloques al azar con siete tratamientos (T): 1) Propiconazol, 2) *Trichoderma* sp., 3) bicarbonato de potasio, 4) mejorador biológico, 5) Clorotalonil, 6) Testigo y 7) Propiconazol + mejorador biológico. Las variables evaluadas fueron el porcentaje de daño de la copa y de la superficie afectada de la hoja. La caída foliar en los pinos se asocia a diferentes agentes, entre los que destacan los micromicetos: *Pestalotiopsis* y *Lophodermium*, así como el insecto *Ocoaxo aff. fowleri*; sin embargo, en los sitios de estudio no se registraron de forma constante. Los pinos con los T4, T3 y T7 presentaron menor porcentaje de caída de follaje. Los resultados indican que el uso de productos biológicos ofrece una alternativa viable para el control del agente causal de la caída foliar del pino; ya que, además, es amigable con el medio ambiente.

Palabras clave: Insecticida, *Lophodermium*, mejorador biológico, *Ocoaxo*, *Pestalotiopsis, Pinus*.

Abstract

Since 2014, different species of pines of *Sierra Norte de Puebla* have shown important defoliations. In the present study, causal agents were characterized and the effectiveness of chemical and biological products for the control of foliar shedding in the pines of *Puebla* was assessed. Three experimental sites were established in the *Tetela de Ocampo* (1) and *Zautla* municipalities (2). An experimental design was applied as random blocks with seven treatments (T): 1) *Propiconazole*, 2) *Trichoderma* sp., 3) potassium bicarbonate, 4) biological enhancer, 5) Chlorothalonil, 6) Control treatment and 7) *Propiconazole* + biological improver. The assessed variables were the percentage of damage to the crown and the affected surface of the leaf. Foliar shedding of pines is associated with different agents, among which fungi genera *Pestalotiopsis* and *Lophodermium* and insect *Ocoaxo aff. fowleri* stand out; however, in the study sites, these agents were not constantly found. The pines with T4, T3 and T7 presented a lower percentage of affectation in the foliage by fall. Results indicate that the use of biological products offers a viable alternative for the control of the agent of foliar shedding of pine, since in addition, it is environment friendly.

Key words: Insecticide, *Lophodermium*, biological improver, *Ocoaxo*, *Pestalotiopsis, Pinus*.

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Introduction

Mexico has an area of 196 437 500 ha, of which, the forest area of *Puebla* covers 1 698 722 ha; 460 771 are forests and 307 455 medium and low warm forests, which represents around 49.4 % of the state territory (Romero, 2011; INEGI, 2014).

The state gathers a great natural richness that comes from its latitudinal location and its altitudinal variation, which gives rise to a large number of ecosystems and types of vegetation, among which coniferous forests stand out (Neyra and Durand, 1998; Yanes, 2011). The main causes of the deterioration of pine-oak forests include deforestation, fires, droughts, pests and diseases, among others (Alvarado *et al*., 2006; Cibrián *et al*., 2007; Romero, 2011). Some of the most recent studies in relation to the above in the entity suggest that they are associated with the presence of insects and fungi (Pérez *et al*., 2016; Castro, 2017).

The main problem is the attack of bark beetles that can kill even healthy trees, followed by parasitic plants; the most important species are *Arceuthobium globosum* Hawksworth & Wiens subsp. *grandicaule*, *A. nigrum* Hawksw. & Wiens and *Arceuthobium vaginatum* (Willd.) Presl. subsp. *vaginatum* (Cibrián, 2011).

On the other hand, one of the micromycetes that has been most recorded as a possible pathogen associated with leaf shedding in the pines of *Puebla* is Lophodermium (Pérez *et al*., 2016). According to Cibrián *et al.* (2007), the fungi of the *Lophodermella, Lophodermium* and *Dothistroma* genera are responsible for the shedding of pines and its distribution covers the forests of Puebla. *Lophodermium* damage is associated with stressors; the infestation begins with Spring and by the end of Summer defoliation occurs. Previously all copies of *Lophodermium* were identified as *L. pinastri* (Shchard.) Chevall., but several species are currently known (Cibrián *et al*., 2007). On the other hand, Dvorak *et al.* (2012) indicated that *Dothistroma* is widely distributed and infects more than 80 species of conifers in the world and that its spread is related to the local climatic conditions.

Therefore, the aims of this research study were to describe the agents that contribute to foliar shedding and to assess the effectivity of biological and chemical products as an option for their control and management.

Materials and Methods

Study area

The study was conducted in *Tetela de Ocampo* and *Zautla, Puebla* municipalities, in three sites (Table 1). The first has an area of 328.80 km^2 and the second of 266.70 km^2; both are located within the zone of temperate climates, with an average annual temperature between 12 and 18 °C; vegetation consists of temperate forests, mainly, where the pine-oak forest (*Pinus* spp.-*Quercus* spp.) and of *táscate* or *junípero* (*Juniperus* spp.) stand out. The most abundant species of conifers is *Pinus pseudostrobus* Lindl. (INEGI, 2014; Pichardo-Segura *et al.,* 2017).

Table 1. Sites, locations and geographical coordinates.

Site	**Locality**	**Municipality**	**Coordinates**	
			Longitude	**Latitude**
1	*Buenavista*	*Zautla*-middle part	97°44’22.12’’	19°44’00.256’’
2	*Buenavista*	*Zautla*-high part	97°37’22.12’’	19°37’00.26’’
3	*Rancho Alegre*	*Tetela de Ocampo*	97º51’29.24’’	19º50’32.26’’

Collection and determination of causal agents

During the establishment of sites and tree selection, tours were made in the affected area to make retrospective observations.

Likewise, foliage samples were taken and placed in hermetically sealed bags for their inspection at the Cenid-Comef Forest Health Laboratory. The needles with lesions were cut into fragments and disinfected with 10 % ethyl alcohol for one minute, with 0.5 % sodium hypochlorite for five minutes and rinsed with sterile distilled water. Subsequently, five fragments were placed in Petri dishes with PDA culture medium (Papa Dextrose Agar, 39 g L^{-1}) and incubated in a Barnstead Lab-Line 3478 oven incubator at a temperature between 25 and 27 ± °C under light / dark conditions: 12:12.

After the fungi developed, they were isolated in order to obtain pure cultures and record their shape, texture, growth time and color of their structures. Semi-permanent preparations were made, with the blue-cotton-lactophenol staining technique, under the following process: a drop of the dye was put on a slide, a sample of mycelium was disintegrated and then, a coverslip was placed.

On the other hand, the samples with ascomycetes in the sexual or teleomorphic phase were processed with the wet chamber technique, which consisted on cutting the fragments of the needles with blights or fruiting bodies, 5 to 6 cm long and placed on a triangle of sterilized glass, in a Petri dish with a filter paper base, moistened with sterile distilled water.

The fractions of needles with signs of foliar damage were incubated for 10 to 12 days, with the aim of inducing the maturation of the fruiting bodies, later with the Carl Zeiss Axiostar Plus stereoscopic microscope, a series of transverse cuts were made to the fruiting bodies, with these they were made semi-permanent preparations and the structures were stained with 1 % blue-cotton-lactophenol, to be observed under a Carl Zeiss Stemi 2000-C optical microscope.

Genus identification was performed with taxonomic keys (Barnett and Hunter, 1972; Nag Raj, 1993; Minter, 1981; Abarca, 2000; Bensch *et al*., 2012; Visagie *et al.,* 2014) and the use of an optical microscope.

Likewise, specimens of insects associated with pine trees were collected during sampling. The specimens were placed in 70 % alcohol bottles with their respective collection labels. They were determined according to the taxonomic group, and copies were compared with the specialized references (Castro *et al.*, 2017; Castro *et al.,* 2019).

Treatment for leaf shedding of pine

Plots were established at each site in which seven trees with signs of foliar damage at a minimum distance of 10 m were selected. The characteristics presented by the individuals were: height from 4 to 7 m, crown diameter from 9 to 21 m and normal DBH (1.30 m), from 200 to 600 cm; with signs of needle lesions (ringed chlorotic spots).

To each pine only one of the following treatments was applied (T): 1) Propiconazole; 2) *Trichoderma* sp.; 3) Potassium Bicarbonate; 4) Biological improver; 5) Chlorothalonil; 6) Control (no product); 7) Propiconazole (on foliage) + biological improver (soil). The levels of aggressiveness of the products used, according to the toxicity scale and threat level of Ramírez *et al.* (2007), went from 2 to 6.

Spray equipment (for foliage or soil) and a low pressure injection system (for trunk) were used for the application. The amount supplied with sprinkler backpacks was prepared according to the crown cover area expressed in square meters; while, for those injected it was considered at a rate of 5 mL per centimeter of DBH. The exact dose and application system for each tree and site are indicated in Table 2.

Table 2. Mode of application and treatment doses in each site for pine leaf shedding control in *Puebla* State.

Site	Number of pines	1	2	3	4	5	6	7
	Treatment	T4	T1	T3	T7	T2	T6	T5
1	Dose (mL)	0.66	105	1.73	1.08	0.83	-	2.45
	Form of application	S*	INY*	A*	S+1.08 A	A	-	A
	Treatment	T1	T2	T7	T5	T3	T6	T4
2	Dose (mL)	117.5	5.81	0.91	4.33	0.86	-	0.96
	Form of application	*INY	*A	*S+0.91 A	A	A	-	A
	Treatment	T7	T3	T4	T6	T2	T1	T5
3	Dose (mL)	1.85	4.56	3.37	-	2.29	98.5	4.25
	Form of application	S+1.85 A	A	S	-	A	INY	A

(*): S = Spray on the ground; A = Spray on foliage, INY = Injection into the trunk.

The first application and sampling was carried out in September 2016; the second supply of products in October (except for trees with the solution injected), in November the third application and, finally, the second data collection, in March 2017.

Analysis of variables

The evaluation of the effectiveness of the treatments was based on the level of damage from two variables: 1) qualitative variable: the color of the needles was considered using the percentage scale proposed by the European Weed Research Society (EWRS) (Champion, 2003) and 2) quantitative variable: it was determined by counting and length (cm) of the chlorotic spots present in the needles of each branch; 10 random fascicles were selected from each one, all chlorotic spots of each of the needles were counted and measured.

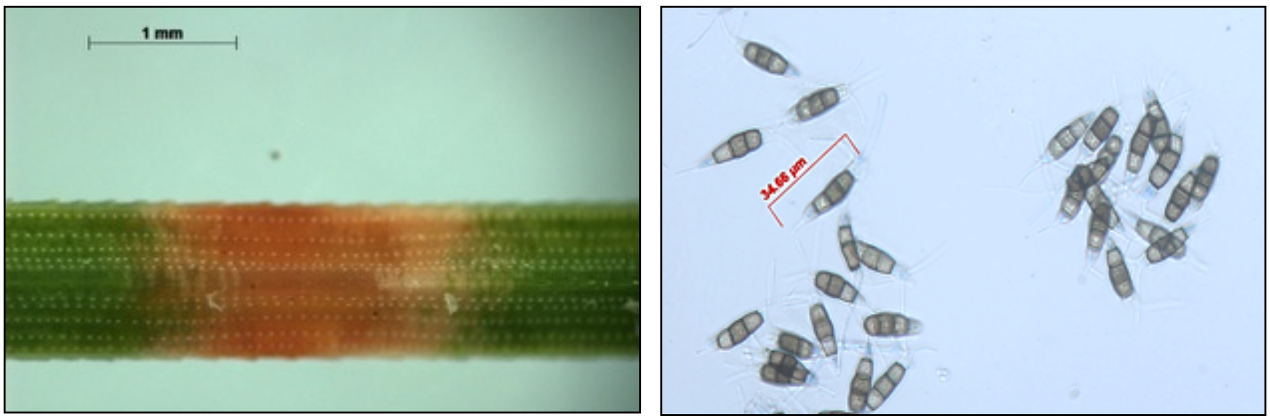
An Excel (2007) base was generated to obtain the descriptive statistics and an average of each treatment for the length of the damage of the chlorotic spots in the acicles. An analysis of variance was performed to determine significant differences between treatments. Dunnett's test was applied for the comparison of multiple means and to compare the effectiveness between treatments of the first and the last sampling Statistic JMP 4 (SAS, 2000).

Results and Discussion

Agents related to leaf shedding

From the foliage samples the corresponding micromycetes were determined up to gender and not species, because it is not the initial focus of this study. The determined micromycetes are: *Alternaria, Aspergillus* sp., *Cladosporium cladosporoides* (Fresen.)*, Penicillium* sp., *Pestalotiopsis* sp*.* and *Lophodermium* sp.; the last two are related as fungi associated with forest tree diseases.

In this study, *Pestalotiopsis* sp. associated with leaf damage in the three experimental sites (Figure 1); in spite of the pathogenic capacity of some species, their development is limited by the vegetative state of the plant; by infecting it, it can directly affect and injure the leaf surface, so it can be considered as a primary causative agent. On the other hand, water splashes are the main link for the dissemination of their spores, so it is suggested as a control measure to reduce or limit sprinkler irrigation whenever possible (Romero *et al.,* 2009).



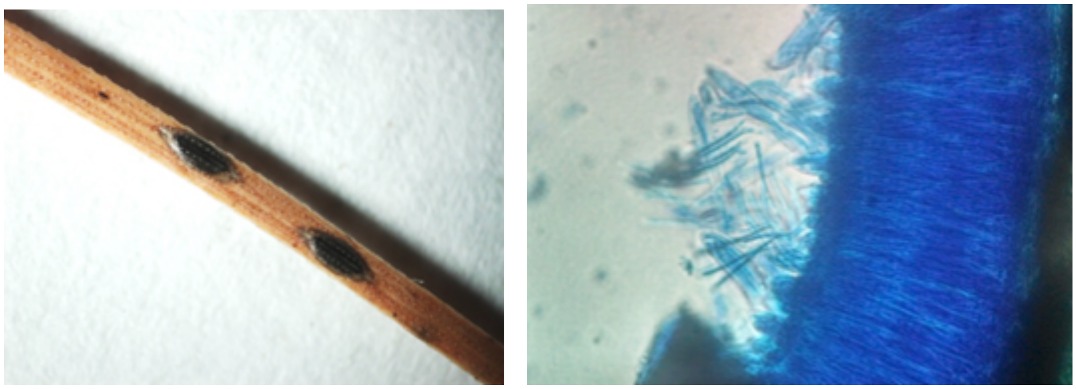
Photogfraph by Muñoz-Belmont.

Left: Chlorotic stain; Right: Structure of the septate and flagellated conidia without mycelium.

Figure 1. *Pestalotiopsis* sp.

Pestalotiopsis was recorded in *Colima* in 2009 associated with "cypress disease" in a 20 ha plantation of *Cupressus lusitanica* Mill. (Valdez *et al.*, 2009). In *Puebla*, according to Morales *et al.* (2017), it has been detected in strawberry crops. Cibrián *et al.* (2007) indicate that *Pestalotiopsis funerea* (Desm.) Steyaert in Mexico affects a large number of hosts, both broadleaved and coniferous.

Lophodermium sp. (Figure 2), like *Pestalotiopsis,* was found in all three sites. According to Cibrián *et al.* (2007), is associated with stressors such as pollution. Meanwhile, Minter (2015) indicates that it is distributed abundantly in various pine forests around the world.



Photograph by Reséndiz- Martínez.

Left = Pine needle with fruiting bodies; Right= Hysterothecium with asci.

Figure 2. *Lophodermium* sp.

Rajkovic *et al.* (2013) indicated that the first visible symptoms of the attack on Christmas trees are yellow spots to brown in the months of September and October, which coincides with the sampling dates of this investigation.

Pérez *et al.* (2016) defined that the climatic requirements for the development of *Lophodermium* exist in *Puebla*, so the pathogen has a great potential to present and spread to new coniferous stands in the state. The micromycete in its stage of growth and development showed a high level of distribution, which suggests that it could become more resistant to changes and alterations of the environment, because after 11 years it is still found and probably increases its population density.

On the other hand, during the tours and sampling the presence of *Ocoaxo aff. fowleri* (Lallemand) in *Zautla* was recorded (Figure 3) which, according to Castro *et al*. (2017) together with *O. assimilis* (Walker) and *O. varians* (Stål) are in the states of *Oaxaca* and *Puebla*, with which the decline of pine forests might be a related.

Photograph by Arriola-Padilla.

Figure 3. *Ocoaxo aff. fowleri* (Lallemand) on pine needles.

The biotic agents identified represent what is normally the relationship between insects and pathogenic fungi, since most diseases that are caused by micromycete species start when the plant is previously damaged or under conditions of physiological stress (Lilja *et al*., 2010), that is, in this case the insect can act as the primary causative agent; however, in the present study, there were no areas with Cercopidae but no affectations in the foliage.

Pichardo-Segura *et al.* (2017) stated that the variations in the values of precipitation, temperature and relative humidity in different locations in *Puebla*, can favor the existence of the causal agents of leaf shedding.

Treatment assessment

The analysis of the qualitative variable (Table 3) for the first site suggests that the treatments of Biological Enhancer, Potassium Bicarbonate and *Trichoderma* sp. they are effective, since the respective treated trees showed slight signs of damage, compared to the control that showed medium damage.

Table 3. Final evaluation of the qualitative variable by visual monitoring of the three sites affected by the causal agent of leaf shedding in *Puebla* State.

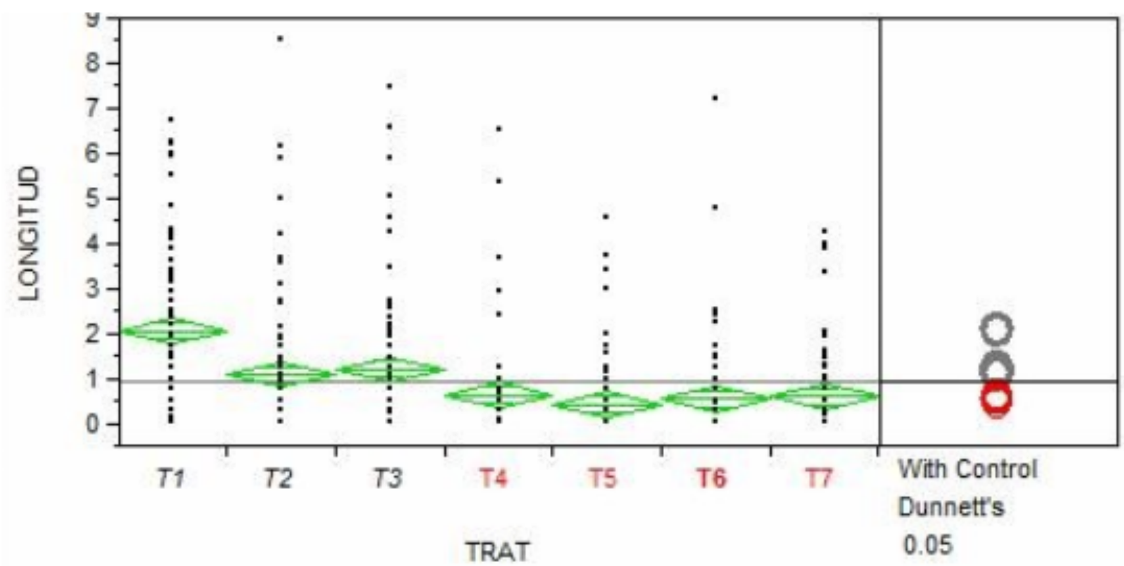
Site	Number of pine	Foliage monitoring	Treatment	Observations
1	1	Very slight signs	Biological enhancer	Lemon green
	2	Intense damages	Propiconazole	Green in PM
	3	Very slight signs	Potassium bicarbonate	Lemon green
	4	Intense damages	Combination	Dry in PA
	5	Slight signs	*Trichoderma* sp.	Dry tips
	6	Medium damage	Control	Dry in PB
	7	Very high damages	Clorotalonil	Dry in PB and PM
2	1	Very high damages	Propiconazole	Dry in PB
	2	Medium damages	*Trichoderma* sp.	Dry in PM
	3	Very slight signs	Combination	Dry tips
	4	Intense damages	Clorotalonil	Lemon green
	5	Intense damages	Potassium bicarbonate	Dry in PM
	6	High damages	Control	Green in PB
	7	High damages	Biological enhancer	Dry in PM and PB
3	1	Very slight signs	Combination	Dry tips
	2	Very slight signs	Potassium bicarbonate	Dry tips
	3	Very slight signs	Biological enhancer	Dry tips
	4	Medium damages	Control	Dry in PB
	5	High damages	*Trichoderma* sp.	Dry in PM and PB
	6	High damages	Propiconazole	Green in PB
	7	Very slight signs	Clorotalonil	Dry tips

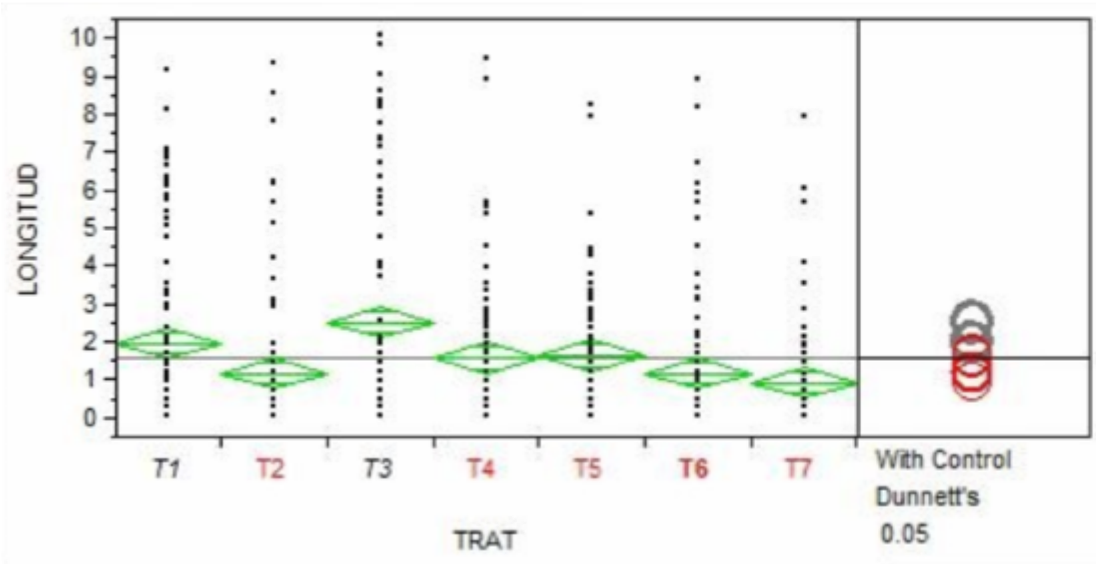
*Crown: PA = High part; PM = Middle part; PB = Low part.

In the second site, the pine treated with Propiconazole + Biological improver, showed very slight signs compared to the witness with very high damage as well as the rest of the pines in the area, where severe damage of the disease was observed from a visit to another in all the trees of the site.

In the third site, the pines treated with Propiconazole + Biological improver, Potassium Bicarbonate, Biological enhancer and Chlorothalonil showed very slight signs compared to the control with medium damage.

The analysis with the Dunnet test among treatments of the first site (Figure 4) suggests that there are significant differences between the means of the last sampling ($p \leq 0.02$). When comparing the results of the first and last data collection, the pine treated with Propiconazole (T1) and the combination of Propiconazole + Biological Enhancer (T7) were the most effective, since the damage between samples remained stable, unlike the rest of the trees that showed a slight increase in damage in the last sampling.





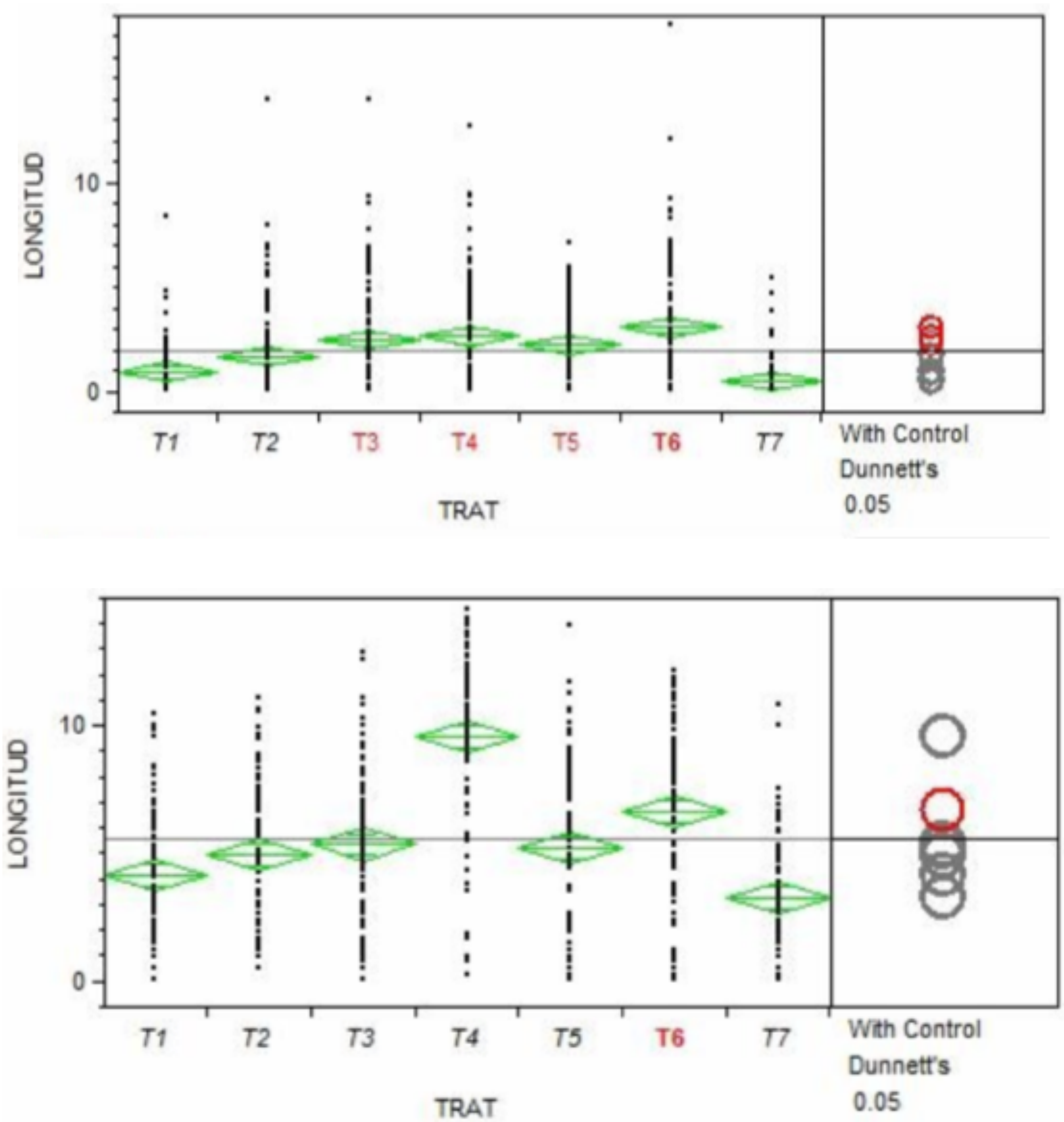
In red treatments below average, and in black above (Dunnett's test).

LONGITUD= Damage length (cm); TRAT = Treatments.

Above = First sampling; Below = Last sampling.

Figure 4. Damage of the needles in Site 1 (*Buenavista, Zautla*-middle part).

The second site (Figure 5) also shows significant differences between the treated pines from the last sampling ($p \leq 0.05$). In spite of the drastic increase in damage in all the pines from one sampling to another, it can be emphasized that the affectation in the pine treated with the combination of Propiconazol + Biological enhancer (T7) in the last sampling, was the lowest compared to the others.



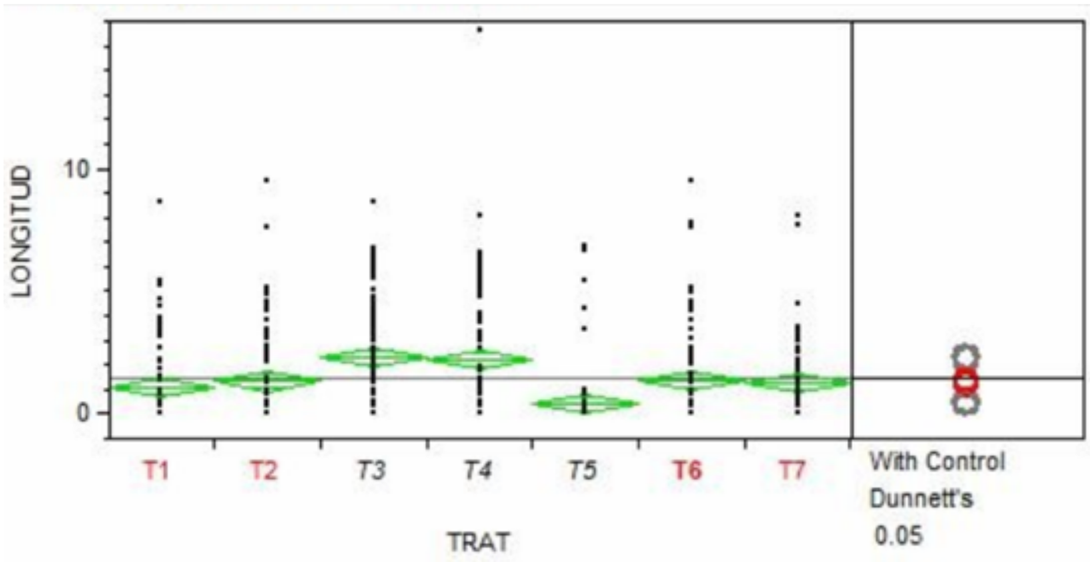
In red treatments below average, and in black above (Dunnett's test).

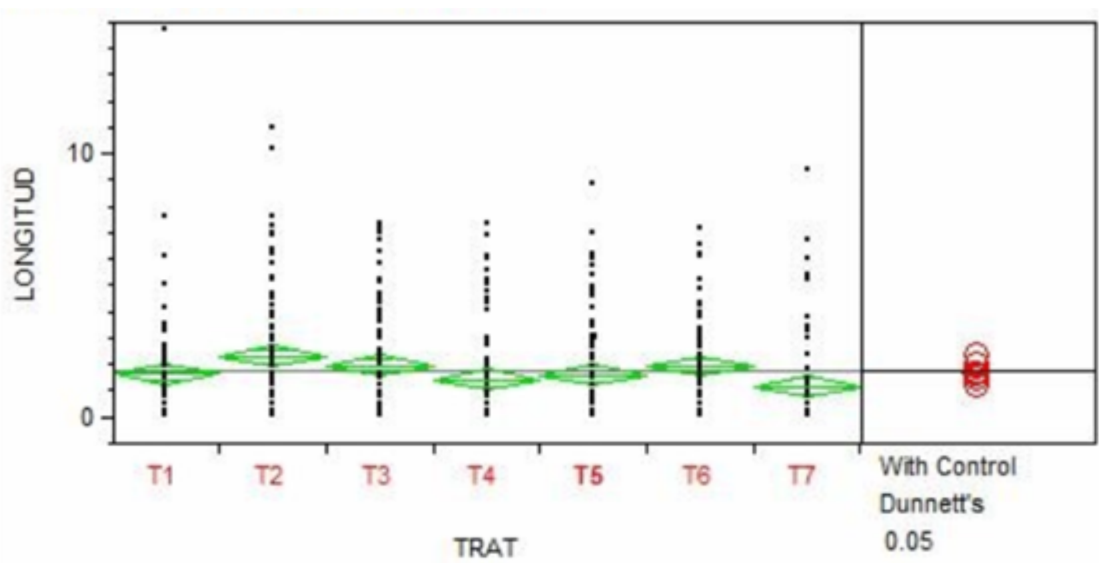
LONGITUD = Damage length (cm); TRAT = Treatments.

Above = First sampling; Below = Last sampling.

Figure 5. Damage of the needles in Site 2 (*Buenavista, Zautla*-middle part).

For the third site (Figure 6), the difference between the treatments of the last sampling reflects a little significant difference ($p \geq 0.05$). However, the shortest length of damage occurred in the tree treated with the combination of Propiconazole + Biological Enhancer (T7).





In red treatments below average, and in black above (Dunnett's test).

LONGITUD = Damage length (cm), TRAT = Treatments.

Above: First sampling; Below = Last sampling.

Figure 6. Damage of the needles in Site 2 (*Buenavista, Zautla*-middle part).

In each of the sites, pines treated with T7 reflected shorter damage length compared to the rest of the pines and between samples; therefore, it was considered the most effective treatment of the study.

Figure 7 shows the pine treated with Propiconazole + Biological improver of the second site (with greater disturbance), during the first and last sampling. The image on the right shows that the surrounding trees show foliage with considerable damage compared to the one treated (T7).

Figure 7. Pine treated with Propiconazole + Biological enhancer of site 2, during the first sampling (left) and last (right).

The combined treatment (Propiconazol + Biological enhancer) indicated to be the most effective according to the analysis of the two variables, which is explained by the protective properties of each product. Propiconazole, being a broad-spectrum systemic fungicide that has a protective, curative effect to the foliage and nutrient additive to the soil, acted not only to recover the pine from the disease, but also provided it with protection in foliage-soil composition of the pathogens present on the site. Therefore, by adding the product of Biological enhancer to the soil, it was probably presented as a double reinforcement, since it is an organic product with beneficial microorganisms that catalyzes, detonates and nourishes both the soil and the foliage, so the combination of products as a single treatment proved to be the most effective among those that were evaluated. Also, although to a lesser extent, the pines treated with Potassium Bicarbonate showed effectiveness in two of the three sites, due to the same protective effect that reduces the susceptibility in the leaves, and provides stability to the whole tree (Zavaleta, 2000).

According to the categories of toxicity and threat to the environment proposed by Ramírez *et al.* (2007), the toxicity level of the biological improver is 2; that of Propiconazole 6 and that of Potassium Bicarbonate of 4, so it is essential to consider this factor for the application of these products in the long term.

Conclusions

The biotic factors identified as a possible origin of the foliar fall in *Puebla* are *Pestalotiopsis, Lophodermium* and Cercopidae; abiotic factors such as temperature, precipitation and pollution favor the stress of trees and the development of causative biotic agents.

Treatments such as the biological improver and the combination of Propiconazole + Biological enhancer were the most effective products against the leaf shedding of pine; the first is less toxic, more friendly to the environment and recommended for natural areas; therefore, it is the most convenient alternative.

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Conflict of interest

The authors declare no conflict of interest.

Contribution by author

Sheyla Nallely Muñoz Belmont: apliccation of treatmients, data collection, micromicetes isolation, writing of the manuscript; Víctor Javier Arriola Padilla: conductance and planning of the project, review of the manuscript; Ramiro Pérez Miranda: selection of the permanent experimental plots, monitoring of the experimental sites and of the affected zones; José Francisco Reséndiz Martínez: micromicetes isolqation and determination; Martín Enrique Romero-Sánchez: data analysis and review of the manuscript.

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