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Presentation

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In Pro-Research, Teaching and Training of human resources committed to Science. The content of the articles and reviews that appear in each issue are those of the authors and does not necessarily the opinion of the editor in chief.

As the first article we present, *Megaesophagus due to persistence of the Aortic Crash*, by ACERO-ORTEGA, Juanita, CAMARENA-MARTINEZ, Rosa Valeria and BAÑUELOS-PINEDA, Jacinto, with secondment at the Universidad de Guadalajara, as the second article we present, Antimicrobial susceptibility of *Staphylococcus Aureus* and *Staphylococcus Coagulasa-negative* isolated from milk of cows with Mastitis from Téjaro, Michoacán, by BEDOLLA, Jose Luis Carlos, LUCIO-Rodolfo and CRUZ-Angel Raul, with affiliation at the Universidad Michoacana San Nicolás de Hidalgo, as the third article we present, *Isolation of entomopathogenic fungi from soils of the Comarca Lagunera as a biological control of pests*, by SÁNCHEZ-MUÑOZ, Salvador[1], PIÑA-BENÍTEZ, Grethel[2], MORENO-GARCÍA, Iris[1] and MARTÍNEZ-VILLALBA, José Antonio[2], with affiliation at [1]Universidad Politécnica de Gómez Palacio & [2]Universidad Iberoamericana Torreón, as the last article we present, *Pteridoflora of the Flora and Fauna Protection Area la Primavera, Jalisco, Mexico*, by COLIN-NOLASCO, Luis Fernando & MACÍAS-RODRÍGUEZ, Miguel Ángel, with secondment at the Universidad de Guadalajara.

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Article **ECORFAN Journal**
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Megaesophagus due to persistence of the Aortic Crash

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Abstract

Within cardiac pathologies a large percentage are of congenital origin in canines, genetics influences, idiopathic megaesophago of unknown, secondary or acquired cause, the megaesophagus presents an abnormal dilatation of the walls, localized or diffuse and other more causes. Megaesophagus presents in a Border Collie canine due to persistent aortic arch. The one-year-old patient had clinical signs compatible with megaesophagus pro-persistence of the aortic arch and died at the time of the physical examination. At the Animal Pathology Research Center, necropsy was carried out with which the Lesions secondary to the presence of the megaesophagus due to persistent aortic arch, and solid food content, an element that contributed to the death of the pet would be negligence with which the owner of the dog was acted on.

Megaesophagus, Persistent aortic arch

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1. Introduction

Among the cardiac pathologies a large percentage are of congenital origin in canines, it is spoken of this disease in which genetics influences, the megaesophagus of unknown cause is called Idiopathic, it can be congenital and manifest in the puppies or affect the adults, the congenital is hereditary in the hard-haired fox terrier and miniature shnauzer, they have racial predisposition the Great Dane, German Shepherd, Labrador retrievers, Shar Pei Chinese Newfoulands and Irish Setter. In the congenital there would be an alteration in the vagal afferent innervation of the esophagus. (hereditary heart disease), environmental factors, Infectious, toxicological, nutritional, etc.

A secondary or acquired megaesophagus is also defined which is caused by many neuromuscular diseases, neuropathies and myopathies. The megaesophagus is characterized by an abnormal dilatation of the localized or diffuse permanent walls of the esophagus with decrease or absence of its function or motor peristalsis of the organ. Several clinical forms of the disease have been reported and described in dogs; an idiopathic congenital form with manifestation of clinical signs before or shortly after weaning; another idiopathic acquired of appearance in the adult age and a secondary form acquired product of a previous primary condition.

Primary causes include segmental extraluminal esophageal obstruction by compression of the esophagus between the abnormal vessels and the base of the heart. The megaesophagus is classified as a partial and total megaesophagus, according to the extent of dilatation in the esophagus. The causes of the partial megaesophagus respond to intrinsic stenosis (scarring) and extrinsic causes such as compressions due to neoplasms of neighboring organs and of vascular origin, for example persistence of the right aortic arch (PAAD).

The total megaesophagus is classified as idiopathic congenital, the etiology of which is paresis or paralysis of the esophagus, and the total megaesophagus acquired, in these cases are unknown. In the presence of this pathology the animal can not swallow in a normal way which is placing the dish on the floor, as the food remains in the esophagus or accumulates in the dilation formed in the esophagus, therefore the recipient should be placed your meal elevated at an angle between the dog's neck and the floor from 45 to 90°.

This is achieved by placing the plate on a chair or table depending on the size of the pet Persistence of the arch (aortic arch). The aorta usually comes from one of the left aortic arches, remaining together with the ductus arteriosus on the same side (left) of the trachea and esophagus. Malformations of the megaesophagus are frequently observed during weaning, which is when the solid or semisolid feeding starts, the dilation increases in size while the time passes, which can occupy a large part of the cranial thoracic cavity.

Physical examination shows malnutrition, delayed development, cachexia. The initial diagnosis based on observation of the physical state, age and size of the dog, should be supported by taking a simple chest plate and contrast, thus confirming the stenosis and thus eliminate the concomitance of congenital generalized esophageal hypomotility.

The esophagus is a tube that connects the throat with the stomach, when food reaches the esophagus by a neurological reflex, the contraction and sequential relaxation of the musculature takes place, leading the food to the stomach for digestion.

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When the esophagus loses its muscle tone completely and dilates, it does not coordinate properly the movements that cause the food to progress towards the stomach, which is why the food tends to slide in the opposite direction to normal within the esophagus according to severity, and is regurgitated through the snout without having been digested, for not having reached the stomach.

Abnormalities of the vascular ring: - These are bands of tissue that compress directly into the esophagus. These bands are basically remains of the fetal blood vessels that normally disappear before birth. In this case, the treatment is surgical (this band is eliminated) obtaining an improvement.

The acquired causes are those that secondarily lead to a megaesophagus are: Myasthenia gravis: - It is considered the most common cause of megaesophagus in dogs.

In this case, the neuro-muscular union is destroyed by the immune system and the muscle signals are not sent by the nervous system to coordinate the esophageal muscle contractions, which is why it does not contract, which causes a secondary megaesophagus.

- Disseminated lupus erythematosus
- Degenerative neuropathies
- Idiopathic
- Esophageal stenosis etc.

2. The case presented is:

A male dog of the Border Collie race of one year of age is presented for consultation in whose clinical history presents regurgitation, dyspnea, anorexia, lethargy, posture of pain, pity, rejection of food depression and fatigue. It has a complete vaccination and deworming calendar, food is based on croquette, tap water, vitamin supplements, normal urination and excretion.

Upon the physical examination, he dies and is sent to the Animal Patology Research Center to perform the necropsy.

2.1 Macroscopic findings

External inspection: good body condition, ocular and oral mucosa with severe pallor, presence of discrete bacterial plaque in teeth.

- Lymph nodes: slightly enlarged, discrete multifocal petechiae.
- Spleen: moderate diffuse congestion, areas with moderate diffuse thickening of the capsule.
- Liver: severe congestion.
- Encephalon: discrete congestion.
- Lung: discrete congestion and diffuse moderate emphysema.
- bronchi: presence of croquette-based food in the light.
- Esophagus: severe dilatation, (megaesophagus), due to the persistence of the arch, from the cranial portion to the middle portion, the presence of abundant, lightly compressed, croquette-based food. Thickened mucosa with diffuse moderate congestion.

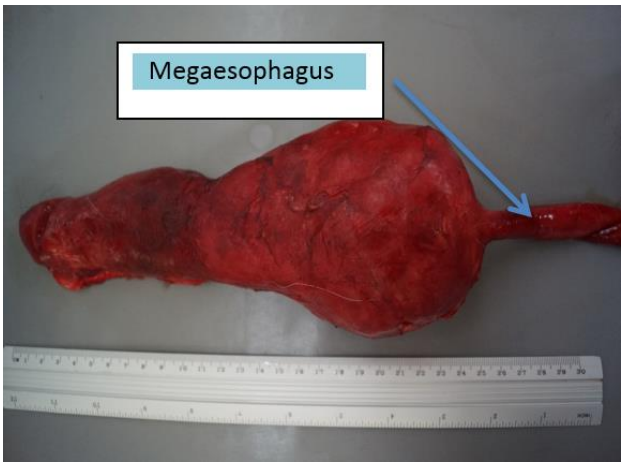

Figure 1

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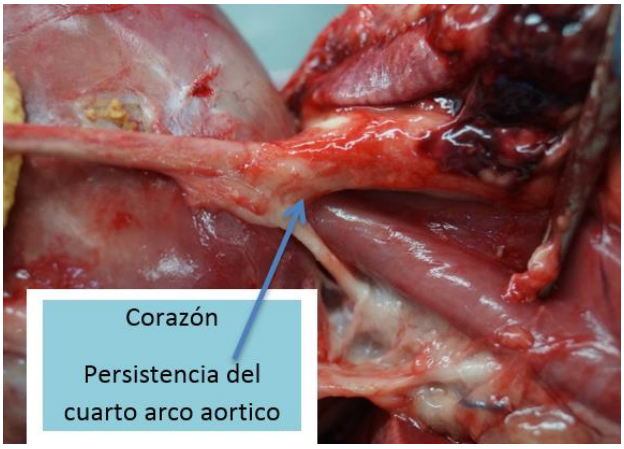

Figure 2

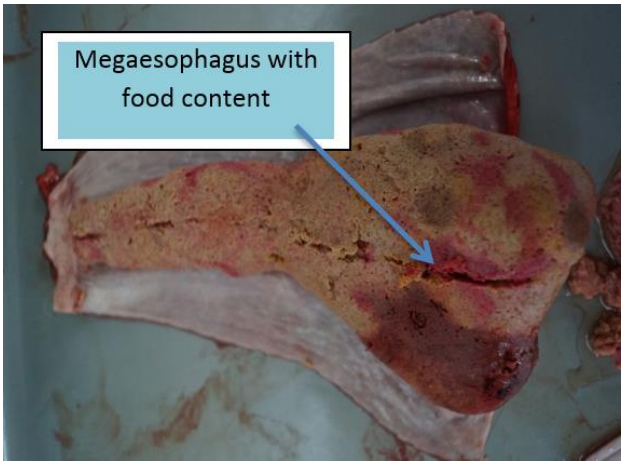

Figure 3

Stomach: Presence of croquette-based food, moderate diffuse congestion and moderate thickening of the walls.

Intestine: catarrhal yellowish content in first portion and greenish in colon, moderate thickening of the walls, moderate diffuse congestion in the mucosa, presence of cestode parasites in the middle part of the jejunum.

Heart: moderate congestion, moderate dilatation of the right ventricle. Persistent aortic arch with the consequent constriction of the esophagus.

Kidneys: discrete congestion in the marrow, whitish areas of radiated appearance in the cortex, detachment of the parenchyma when the capsule is removed.

3.-Discussion

The canine was treated with negligence on the part of the owner, these cases attended to early, are subjected to a surgical process and increases their life expectancy in 85 to 92% of the cases of pets that present it.

4.-Conclusions

The megaesophagus is not always fatal, everything depends on the time it is diagnosed and treated, in addition to taking adequate measures for feeding and handling the pet.

The persistence of the aortic arch has several origins among which are hereditary, by alteration of several genes (polygenic recessive). Veterinary medicine professionals should be alert before breeds predisposed to this disease, and as far as possible not to allow breeding among siblings, or parents and children mainly in breeding sites of these breeds the offspring will present problems after weaning. In this case I contribute the negligence of the owner, because his attention was very late, for which it was not possible to save him.

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Article **ECORFAN Journal**
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Antimicrobial susceptibility of *Staphylococcus Aureus* and *Staphylococcus Coagulasa-negative* isolated from milk of cows with Mastitis from Téjaro, Michoacán

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Abstract

The aim of this study was to determine the antimicrobial susceptibility of *Staphylococcus aureus* and coagulase-negative staphylococci isolated from milk of cows with subclinical mastitis in Téjaro population, Michoacán. This work was conducted of September to January of 2013 in 17 dairy herds Téjaro, Michoacán, which have an average of 8 cows per stall. Subclinical mastitis was determined by testing according to California Wolter *et al.* (2004), 408 samples were obtained from 102 cows. Antibiotic susceptibility testing was performed on 102 strains of staphylococci (41 S. *aureus* and 61 coagulase-negative staphylococci) isolated from milk samples were obtained from cases of subclinical mastitis in dairy herds Téjaro Michoacán. We conclude that penicillin and ampicillin were the antibiotics that had higher resistance *Staphylococcus aureus*, but not coagulase-negative staphylococci, which showed increased resistance to erythromycin and tetracycline.

Antimicrobial susceptibility, Staphylococcus *aureus*, Coagulase-Negative Staphylococci, Subclinical mastitis

Citation: BEDOLLA, Jose Luis Carlos, LUCIO- Rodolfo and CRUZ-Angel Raul. Antimicrobial susceptibility of *Staphylococcus Aureus* and *Staphylococcus Coagulasa-negative* isolated from milk of cows with Mastitis from Téjaro, Michoacán. ECORFAN Journal-Republic of Guatemala. 2017, 3-4: 6-9.

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Introducción

Staphylococcus aureus are the main object of study on antimicrobial resistance in subclinical mastitis due to its importance and the high frequency of isolation of strains resistant to methicillin (García, 2005). However, at present the incidence of mastitis caused by Coagulase Negative Staphylococci (NEC) has increased substantially (Torutoglu et al., 2006), due to poor handling, hygienic conditions and control of contagious pathogens in bovine farms. Staphylococci also develop resistance to certain antimicrobials such as Erythromycin, Ampicillin, Penicillin and Tetracyclines, as they participate in the most common infections in herds with subclinical mastitis.

The tests of susceptibility to these antimicrobials in Coagulase Negative Staphylococcus species, are due to the increase in resistance to Penicillin and Erythromycin when applied in the treatment of herds of cattle (Álvarez et al., 2008), whose purpose is to face the problem of the inadequate use of antimicrobials applied against Staphylococcus aureus and Coagulase Negative Staphylococci.

Objective

The objective of this study was to determine the antimicrobial susceptibility of Staphylococcus aureus and Coagulase-negative Staphylococci isolated from cow milk with subclinical mastitis in the population of Tejaro, Michoacán.

Material and Method

The present work was carried out during the period from September 2012 to January 2013 in 17 dairy farms in Tejaro, Michoacán, which had an average of 8 cows per stable. Subclinical mastitis was determined by the California test according to Wolter et al. (2004), 408 samples of 102 cows were obtained.

The antimicrobial susceptibility test was carried out in 102 staphylococcal strains (41 S. aureus and 61 Coagulase Negative Staphylococci) isolated from milk samples obtained from the cases of subclinical mastitis of the dairy herds of Tejaro Michoacán. The identification of staphylococci was carried out according to Sears and McCarthy (2003), that is, through their colonial morphology, catalase test, Gram stain, coagulase test, mannitol gelatin test and hemolysis.

The antimicrobial susceptibility test was carried out using the disk diffusion method in Agar Mueller-Hinton (Oxoid) according to Torutoglu et al. (2006). Ten colonies placed on blood agar medium, and incubated at 37 ° C for 18 hours, were suspended in 2 ml of sterile saline at a density approximately equal to the density of standard 0.5 of McFarland. A dry sterile cotton swab was placed in the suspension, then the excess broth was removed by pressing and turning the swab against the inside of the tube.

The bacterial suspension was inoculated onto the Mueller-Hinton agar with the sterile swab in such a way that the entire surface of the agar was covered. Subsequently discs containing the following antimicrobials: ampicillin (Bio-Rad, 10 μg), cephalothin (Bio-Rad, 30 μg), cefotaxime (Bio-Rad, 30 μg), ceftazidime (Bio-Rad, 30 μg), cefuroxime (Bio-Rad, 30 μg), Dicloxacillin (Bio-Rad, 1 μg), erythromycin (Bio-Rad, 15 μg), gentamicin (Bio-Rad, 10 μg), pefloxacin (Bio-Rad, 5 μg), penicillin (Bio-Rad, 10 U), tetracycline (Bio-Rad, 30 μg), and trimethoprim / sulfamethoxazole (Bio-Rad, 25 μg) were placed on the surface of the medium and incubated aerobically at 37 ° C for 18 hours.

The results were recorded as sensitive or resistant by the diameter of the inhibition halo according to the interpretative standards of the NCCLS. The reference strain used for the antimicrobial susceptibility assays was ATCC 25923 of S. aureus.

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Results and Discussion

Next, information on the in vitro activity of 12 antimicrobials against strains of staphylococci isolated from subclinical bovine mastitis is presented. The antibiotic resistance rates of the strains isolated from S. aureus and ECN from bovine mastitis are detailed in table 1, in which it is observed that S. aureus and NEC isolates present a high rate of resistance to penicillin and ampicillin. and slightly to erythromycin.

The sensitivity of staphylococci isolated from milk of cows with mastitis to select antimicrobial agents has been previously reported (Gooraninejad et al., 2007). Sensitivity to penicillin predicts sensitivity to other β-lactam antimicrobial agents such as ampicillin. In this study 29.2% (12) of the strains isolated from S. aureus were resistant to penicillin, 21.9% (9) to ampicillin, 9.7% (4) to erythromycin and only 2.4% (1) of the strains they were resistant to tetracyclines. Regarding the sensitivity of strains of S. aureus, it was found that 100% (41) of these were sensitive to cephalothin, cefotoxin, cefepine, cefuroxime, dicloxacillin, gentamicin and trimethoprim / sulfamethoxazole.

This result of resistance to penicillin is below that reported by Bezek, (1998) in a study conducted with cows with mastitis in the United States (44%), by Gentilini et al. (2002) in Argentina (40%), by Malinnowski et al. (2002) in Poland (66.7%) and by Mylus et al. (1998) in Finland (50%).

Regarding ECN strains, 21.3% (13) of these were resistant to erythromycin, 13.1% (8) to tetracyclines, 11.4% (7) to penicillin, 8.1% (5) to ampicillin, 8.1 % (5) to gentamicin, 11.27% (7) were resistant to trimetropin / sulfamethoxazole, dicloxacillin, cefotoxin, cefepime and cefuroxime respectively. However, 100% (61) of strains isolated from ECN were sensitive to cefalotin and levofloxacin.

In other studies conducted on penicillin resistance by NEC, it was found that these results are also below what was found in Argentina (30%) by Gentilini et al. (2002), Finland (37.2%) by Myllus et al. (1998) and Denmark (36.1%) by Aarestrup et al. (nineteen ninety five).

This resistance to penicillin observed in this study as pointed out by Torutoglu et al. (2006) may be because this antibiotic represents the main antibiotic recommended for the treatment of mastitis in cows. Therefore, it is concluded that Staphylococcus aureus were the ones that showed greater resistance to Penicillin and Ampicillin, but not Coagulase Negative Staphylococci, which showed greater resistance to Erythromycin and Tetracyclines.

These differences observed in the activity of antibiotics used against staphylococci show the importance of antibiotic susceptibility tests for the identification of bacterial agents. So in the treatment of animals infected by these bacteria, it is important to determine through an antibiogram the resistance they present to antibiotics and thus apply the appropriate treatment.

Conclusions

It is concluded that Penicillin and Ampicillin were the antibiotics to which Staphylococcus aureus showed greater resistance, but not Coagulase Negative Staphylococci, which showed greater resistance to Erythromycin and Tetracyclines.

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Article **ECORFAN Journal**
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Isolation of entomopathogenic fungi from soils of the Comarca Lagunera as a biological control of pests

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Abstract

The main objective of this study was to isolate entomopathogenic fungi with the ability to degrade exoskeleton chitin from insect pests as the only source of carbon. The fungi isolated in soils from La Comarca Lagunera were inoculated in minimum salt medium (MMS) supplemented with shrimp cuticle (1%). From 14 strains isolated, only 8 showed entomopathogenic activity, presuming the presence of genera *Beauveria* and *Metarhizium*, identified by microscopy with lactophenol blue. In order to corroborate the enzymatic and entomapatogenic activity, the isolates were cultivated in MMS in the presence of domestic pests from the northern region of this country (*Acheta domesticus*, *Blatella germanica* and Musca domestica) showing activity at 72 h^{-1} and having their best yields at pH 4. The results obtained in the increment of biomass with the strains AG4 and VE (yields of 70 and 52% respectively) in colloidal chitin at 72 h^{-1} may be the precursor of strategies for biological control of pests in this region.

Entomopathogenic Fungi, Biological Control, Pests, Chitin

Citation: SÁNCHEZ-MUÑOZ, Salvador, PIÑA-BENÍTEZ, Grethel, MORENO-GARCÍA, Iris and MARTÍNEZ-VILLALBA, José Antonio. Isolation of entomopathogenic fungi from soils of the Comarca Lagunera as a biological control of pests. ECORFAN Journal-Republic of Guatemala. 2017, 3-4: 10-22

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1. Introduction

The use of pesticides and insecticides for the control of pests, in favor of the protection of crops, had been the commonly used and useful strategy, but it has also caused damage to health and the environment. There are around 15,000 species that are considered pests, of which 300 species require a timely form of control (Tarek Mohamed, 2002).

The indiscriminate use of pesticides has caused that many insects and mites have become pests much more difficult to control before the use of these synthetic insecticides. This has caused a serious problem in sectors such as horticulture, forestry and agriculture. The environmental and social costs associated with the use of pesticides, reaches about 8 billion dollars each year (Osteen et al., 1993). In a study conducted by the WHO (1990) (cited by Eddleston et al., 2002) between 500,000 and 1 million people are poisoned with chemical pesticides annually and approximately 5,000 to 20,000 lose their lives.

At present, a strategy that is being implemented for the indiscriminate use of pesticides is the application of biological agents; for example, viruses, bacteria, protozoa and fungi. A viable alternative for this problem is the use of entomopathogenic fungi. These microbial agents have a great potential as controlling microorganisms, constituting a group with more than 750 species disseminated in the environment (Díaz et al., 2006). Among the main species of entomopathogenic fungi are Beauveria bassiana, Metarhizium anisopliae, Isaria fumosorosea, Paecilomyces spp, Verticillium lecanii, Smittium morbosum, among others. The majority of these fungi belong to the genera of Chytridiomycetes, Deuteromycetes, Ascomycetes, Zygomycetes, and Oomycetes, found to a large extent in nature (Gul et al., 2014).

For several years, these fungi have been shown to be an important technique for effective management in the biological control of pests. The mechanism of action of entomopathogenic fungi is given by a balanced interaction between the fungus, the host and the environment. The life cycle includes an infectious sporulation stage that germinates in the host's cuticle, forming a germ tube that penetrates the cuticle and invades the insect. The potential increase of infectious spores generates the production of toxins that end the life of the insect (Hajek and St. Leger, 1994).

In this context, it is important to generate new alternatives that favor the indiscriminate use of pesticides and insecticides. Entomopathogenic fungi are a good strategy since there is a wide variety of these microorganisms, they can be easily cultivable in the laboratory and can survive in a parasitic or saprophytic manner in the host.

The objective of the present work was to isolate and cultivate different soil fungi to carry out bioassays with shrimp cuticle, Acheta domesticus, Blattella germanica and Musca domestica with the purpose of analyzing their quitinolitic capacity and alternative biological control to the use of pesticides and insecticides.

1.1 Justification

Currently, it is essential to avoid further deterioration and pollution to the environment. In the area of agriculture, few ecological alternatives have been implemented to help this problem. One of the main strategies is the control of pests through the use of biological agents. The implementation of entomopathogenic fungi in agriculture potentially helps to reduce the adverse effects produced by the indiscriminate use of pesticides, both for the environment and for the human being.

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The carrying out of integrated pest management is of vital importance for insects to be combated by means of natural enemies that can: prevent damage to the ecosystem, decrease the economic resources allocated for that purpose, and not alter the chemical composition of the food, develop a sustainable agriculture and not cause harm to the health of people.

In addition to the above, the cost-benefit can be obtained in the short term, because it can reduce the use of conventional chemicals without minimizing the production of the harvest.

1.2 Problem

At present, the issue of environmental pollution is becoming stronger. Population growth has caused an excessive demand for food, including fruits and vegetables. To supply these food needs, the use of pesticides is the main alternative for the protection of crops, these substances have been the strategy commonly used, but also, they have caused negative impacts to the environment, due to excessive and indiscriminate use.

Pesticides have been shown to be related to health problems in humans, the residence time they can have in soil and water is considerable, they can bioaccumulate or biotransform into compounds more toxic to humans and the environment.

Likewise, the inappropriate use and excessive application of these chemical substances brings about the deterioration of the environment caused by the direct application to the crops, their incorrect storage and accidental spills, so that, these substances are dispersed in the environment affecting the the biotic and abiotic factors of the ecosystem.

So it is convenient to find new strategies to solve these environmental and public health problems. One of the most viable are entomopathogenic fungi; which, they stand out for having the metabolic ability to infect insects and cause their death.

The use of biopesticides based on this type of microorganisms helps to reduce the environmental impact coming from the pesticides, they are selective and show a high specificity. Likewise, they have the ability to multiply and spread their spores in the environment, producing an important defense mechanism to the pest. These characteristics make the entomopathogenic fungi a viable strategy for the biological control of pests.

1.3 Hypotesis

– Research hypothesis: Fungi isolated from different sites of the Comarca Lagunera have the capacity to degrade insect pests in the region.

– Alternative hypothesis: Fungi will show better growth and greater chitinolytic ability at acid pH.

– Null hypothesis: Expression of extracellular proteins in MMS media enriched with colloidal chitin will not be proportional to the amount of reducing sugars.

1.4 Objectives

1.4.1 General objective

Analyze the degradative potential in insects that have entomopathogenic strains isolated from different soils of the Comarca lagunera as an alternative to the biological control of pests.

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1.4.2 Specific objectives

- Isolate entomopathogenic fungi from humid soils of the region of the Comarca Lagunera.
- To determine the ability of isolated fungi to hydrolyze chitin by bioassays with shrimp cuticle and biomass generation.
- Analyze the chitinolytic activity by a colorimetric technique.
- To evaluate the pathogenicity of the strains implementing bioassays with insects considered pests in the region.

2. Theoretical framework

Biopesticides based on different microorganisms are considered good control agents for harmful insects for agricultural crops. For example, the use of entomopathogenic fungi in the biological control of pests has increased globally in recent decades, yielding good results (Faria and Wraight, 2007).

There are two ways in which an affectation by the fungus to the insect can happen, one is the chitinolytic activity that the fungus has and the second is through the production of toxins. The mechanism of pathogenicity of both processes of these fungi has been studied in such a way that currently the main stages of the infective process are already known; Thanks to this, strategies have been established to improve its application as biological control.

Entomopathogenic fungi unlike other entomopathogenic agents have unique mechanisms of action since they do not need to be ingested by the insect to control it, but it infects it by contact and adhesion of the spores to different parts of the body of the insect, causing vital damage to the pathogen (Ortiz-Urquiza and Keyhani, 2003).

From a general perspective fungi present the following development phases as mentioned by Alean Carreño (2003), the mechanism of action is divided into three phases: The first phase consists of the adhesion and germination of the spore to the surface of the exoskeleton of the insect. In this process there is an interaction between the hydrophobins found in the conidium and the hydrophobic surface of the exoskeleton. Subsequently, penetration takes place in the second, a process in which, according to the species of fungus, a mucilaginous substance is formed that holds it together to form a germinal tubule that penetrates through the pores and outer layers of the epicuticle. Finally, the last step is the growth of the fungus. Which usually results in the death of the insect.

The death of the insect occurs more quickly when it is affected by a fungus that produces considerable amounts of toxins. Among these toxins are the destruxinas (demetildestruxina and protodestruxina). Its mode of action is to inhibit the synthesis of DNA, RNA and proteins in the cells of insects (Pucheta et al., 2006); in addition, they are substances of low toxicity, but of much virulent activity on insects, mites and nematodes.

Everything described above favors the organic composition of the exoskeleton of insects. The cuticle of the insects consists of crystalline chitin nanofibers, some of which are deacetylated and play an important role, since they allow the formation of resistant and support tissues for arthropods, arachnids, insects, nematodes, annelids, brachiopods, mollusks, among others. Also this carbohydrate is part of the cell walls of some fungi; as ascomycetes, basidiomycetes, which are also composed of glucans and other polymers (Ezekiel et al, 2014). Chitinase is an enzyme that can hydrolyze chitin and has been found not only in fungi, but also in bacteria, plants, vertebrates and invertebrates.

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They have an important role in the process of molting of insects, the digestion of chitinous foods and as a defensive enzyme against chitinous pathogens. Entomopathogenic fungi synthesize metabolites that are toxic against insects and, generally, are synthesized when the fungus has penetrated the exoskeleton (Téllez-Jurado et al., 2009).

The entomopathogenic fungi provide excellent solutions for biological control. The panorama is very promising and this can be extended to find uses as in the field of agriculture and, in this way, to develop an integrated management of insects considered pests and fungi as part of a sustainability that contributes to reduce the effects of environmental pollution. The deciphering adequately the mechanism of action of enzymes and toxins, will allow an important step to the development, generation and implementation of bioinsecticides, developing research in the field of biotechnology, agricultural and environmental.

3. Materials and methods

3.1 Sampling

A total of 6 samples were collected from humid soil from different points of the urban area of the Comarca Lagunera under aseptic conditions. Each sample contained approximately 10 g of soil deposited in sterile polyethylene bags. These samples were stored at 4 ° C for later use (Feng and Yang, 2010).

3.2 Isolation and cultivation of fungi

The samples were isolated by the technique of serial solutions using as medium Papa Dextrose Agar (PDA); to which 1% gentamicin was added to prevent the growth of bacteria. After 72 hours of incubation at 27 ± 2 ° C, a significant growth of fungal colonies was observed. The individual colonies were sub-cultivated and stored on agar inclined with PDA and antibiotic at 4 ° C for their conservation.

This procedure was repeated three times in order to have isolated colonies with entomopathogenic fungal characteristics (García et al., 2011).

3.3 Morphological characterization

To morphologically characterize the isolated fungi were stained with the reagent Lactophenol Cotton Blue (LPCB) and were observed under a microscope at 100X (Hamiduzzaman et al., 2012). For the macroscopic and microscopic characterization of the isolated species, the criteria established in their research by Mier et al. (2002) were taken into account, including colony color, consistency, surface area, growth rate, appearance, size and hyphae.

3.4 Bioassays with shrimp cuticle

The isolated fungi were planted in a minimal medium of salts with colloidal chitin as the sole carbon source. First, colloidal chitin was prepared using dry shrimp. The shrimp was washed with distilled water and placed in 2N HCl for 24 hours. Afterwards, it was washed, dried to detach the cuticle and macerated to obtain a fine powder that passed through a 40 mesh screen. It was stored at 4 ° C until its later use (Castro et al., 2011).

Then, a minimum salt medium (MMS) was prepared (2.0 g of KH_2PO_4, 0.4 g of $CaCl_2 \cdot H_2O$, 0.3 g of $MgSO_4 \cdot 7H_2O$, 37 mg of $CoCl_2 \cdot 6H_2O$, 50 mg of $FeSO_4 \cdot 7H_2O$, 16 mg of $MnSO_4 \cdot H_2O$, 14 mg of $ZnSO_4 \cdot 7H_2O$ for 1 L) (Jackson et al., 1997) with 1% of Gentamicin, enriched with a concentration of 1% colloidal chitin. Treatments were carried out with pH 4, 5 and 7 with a final volume of 50 mL. The pH was adjusted with H_3PO_4 and NaOH. Finally, the medium was distributed in glass jars in which the previously obtained strains were inoculated in triplicate. They were incubated at 27 ± 2 ° C for a minimum of 72 hours or until mycelial growth was observed.

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3.5 Determination of biomass

To analyze the biomass growth of the fungi, the dry weight was determined according to the methodology proposed by García-Gutiérrez et al. (2013). First, fungi were allowed to grow for 72 hours in MMS with shrimp cuticle. Subsequently, aliquots of 5 mL were taken to determine the biomass produced by the dry weight technique.

The samples were filtered through a WhatmanTM No. 42 filter paper, previously dried at 75 ° C for 24 hours. Subsequently, they were cooled in a desiccator at room temperature and the filters were weighed, obtaining the grams of the fungus. This process was carried out in an initial and final stage of the research process at pH 4, 5 and 7 in triplicate.

3.6 Determination of chitinolytic activity

To determine microorganisms with positive chitinolytic capacity, a basal medium containing 0.3 g of MgSO4 • 7H2O, 3.0 g of (NH4) 2SO4, 2.0 g of KH2PO4, 1.0 g of citric acid monohydrate, 15 g of agar, 200 g was prepared. µl of Tween-80, 4.5 g of colloidal chitin and 0.15 g of bromocresol purpura (for 1 L of final volume).

The pH was adjusted to 4.7 and autoclaved at 121 ° C for 15 minutes. The medium was emptied into Petri dishes for polymerization. To check the chitinolytic activity, the isolated strains were inoculated with digralsky loop in the medium. They were incubated at 25 ± 2 ° C for 3 days to see the formation of colored zones. Trials were conducted in triplicate (Agrawal and Kotasthane, 2012).

3.7 Determination of reducing sugars and crude protein

To determine reducing sugars, a calibration curve was made with different concentrations of dextrose (100-1000 mg / L), these were reacted in a 1: 1 ratio with 3,5-Dinitrosalicylic acid (DNS), then heated to a bath Maria for 10 minutes and measured at a wavelength of 545 nm (Miller, 1959). The Bradford (1976) trial was used to measure the amount of crude protein intra and extracellular. A standard curve was made with albumin (100-500 mg / L).

The reaction was performed with the following ratio 1: 7: 2 (sample: dH2O: Bradford reagent) and measured at a wavelength of 595 nm. The determination of reducing sugars was evaluated at the beginning and end of the process, the protein was evaluated every 24 h-1.

3.8 Bioassays with insects

To check the entomopathogenic capacity of the fungi, some modifications were made to the protocol proposed by Gutiérrez and collaborators in 2014. The bioassays were elaborated with MMS and insects that are considered common pests in the region. Insects were obtained such as Acheta domesticus, Blattella germanica, and Musca domestica; which were previously washed with a 5% sodium hypochlorite solution to disinfect them and, before the medium solidified, they were added to the medium. 100 µL of the fungus adjusted to 0.5 was inoculated on the McFarland scale. Plates were incubated at room temperature until mycelial growth was observed.

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4. Results and discussion

4.1 Isolation and cultivation of fungi

Of the 6 sites sampled, a total of 14 isolated strains were obtained; of which, only 8 were those that showed ability to adapt better to the minimum salt medium with shrimp cuticle. The strains (AM, VE, NE, AG1, AG2 and AG4) had mycelial growth after 72 hours of incubation. On the other hand, strains AG3 and AG5 showed a slow adaptation to MMS and a mycelial growth was observed after 96 hours. The experiment was carried out in triplicate and the strains were reseeded at least 3 times until macroscopic typologies characteristic of the fungus were obtained.

4.2 Morphological characterization

The morphological characterization was performed by the differential technique of lactophenol blue. The microscopic features of hyphae and vegetative mycelia were determined to identify genera of fungi with entomopathogenic properties (Beveridge et al., 2007).

The isolated strain AG1 was identified by the genus Beauveria. This strain was characterized by a slow and circular growth of white color for 10 days at room temperature. The colonies were approximately 20 mm in diameter, their appearance was woolly and at first the colonies turned yellow. Likewise, it presented a soft texture with a flat surface, similar to what is established by Domsch et al. (1993).

In section A of figure 1 the strain AG2 is shown, which shows the morphological characteristics of the genus Metarhizium. It was observed under the microscope that it has cenocitic hyphae and the olive green color was well defined in the culture. Its growth was a little faster reaching 38 mm in diameter in 10 days at room temperature.

The fungal colonies grew in a circular manner with cottony appearance, presented a flat surface and a soft texture (Domsch et al., 1993, Mier et al., 2002). With respect to the other isolated fungi, it is even more pretentious to ensure the identity of their genus by its macroscopic and microscopic morphological.

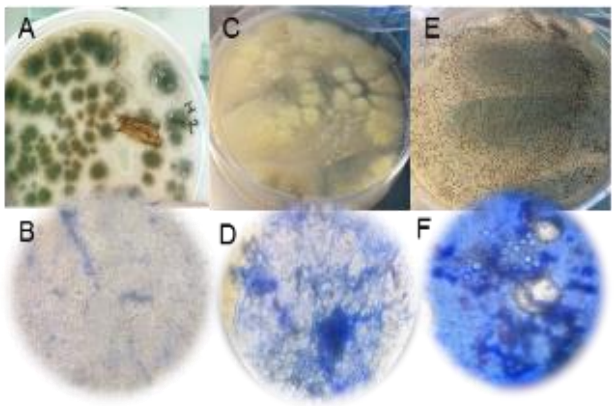

Figure 1 Isolated strains of entomopathogenic fungi from moist soils. A) Cultivation fungus Metarhizium, B) microscopy at 100 X of the fungus Metarhizium, C) Culture fungus Beauveria, D) microscopy at 100 X of the fungus Beauveria, E) Culture of the isolated strain AG5 and, F) microscopy at 100 X of the fungus AG5.

4.3 Bioassays with shrimp cuticle

75% of the strains showed a mycelial growth close to 72 hours after being inoculated in MMS with colloidal chitin as the sole carbon source. The rest of the fungi obtained a visible macroscopic growth after four days of incubation at room temperature. Being NE, AM and AG5 those that presented these characteristics. The size of the strains ranged between 10-40 mm in diameter (Figure 2).

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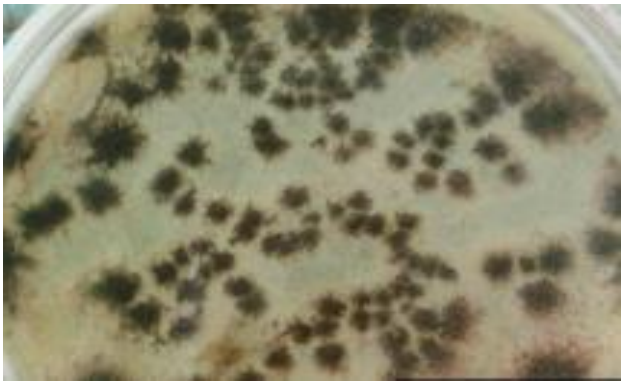

Figure 2 Growth of isolated NE fungus in MMS with colloidal chitin as sole carbon source

There is evidence that fungi that grow under chitin conditions as the only carbon source and with the presence of ammonium salts as an inorganic source of nitrogen, generate metabolites, including enzymes, that favor their reproduction and hydrolysis of β1-4 bonds. of the N-acetylglucosamine units (Shanmugaiah et al., 2008).

4.4 Determination of biomass

The biomass production was measured after 10 days of growth. This analysis allows to identify the strains that showed the highest growth in the MMS medium with colloidal chitin as the sole carbon source (table 1). The AG4 strain inoculated at pH 4 obtained the highest yield (70%) at pH 4 and 25% at pH 5 and 7. Likewise, the fungus referenced as VE increased its biomass by 52% at pH 7 and 24% at pH 5. On the other hand, strains NE (pH 5 and 7), AG3 (pH 4 and 5), and AG5 (pH 4, 5 and 7), had a lower yield in biomass production; which, grew below 20%.

The growth in biomass of the fungi is related to the ability to capture the organic nitrogen present in the environment. Nitrogen favors hyphal reproduction (Eyal et al., 1994) and this can proceed by hydrolyzing the chitin present in the insect as a source of carbon and nitrogen.

4.4 Determination of chitinolytic activity

The basal chitinase detection medium was supplemented directly with colloidal chitin (4.5 g / l) and bromocresol purple dye (0.15 g / l) (Agrawal and Kotasthane, 2012). The result was a bright yellow color. When chitinolitic activity occurs, the medium turns to colorations between purple and pink.

In figure 3 some of the strains that showed good chitinolytic activity are shown. The color turn began at approximately 72 hours of incubation at room temperature. Isolated strains AM, NE and VE showed the highest chitinolytic activity after 10 days of incubation, compared to the rest. What is significant is a slow adaptation to the basal medium with colloidal chitin as a carbon source, because in the beginning the degradation of chitin is complex and slow.

pH	Strain	% biomasa
4	AM	43
7	AM	45
4	NE	39
5	VE	24
7	VE	52
5	AG1	28
7	AG1	25
4	AG2	32
5	AG2	28
7	AG2	29
7	AG3	32
4	AG4	70
5	AG4	25
7	AG4	25

Table 1 Percentages of most significant biomass yields of isolated fungi

This is indicative that fungi excrete some enzymes amylases, proteases and chitinases that are related to the pathogenicity of the fungus, specifically in the part of adhesion and germination of spores (Chernin et al., 1997).

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Other research indicates that the chitinolytic activity is directly related to the enzymatic expression of the fungus to the extracellular medium. This would allow to degrade the exoskeleton of the insect by releasing an enzymatic complex that includes hydrolytic enzymes, among which stand out, the chitinases (Michel et al., 2005).

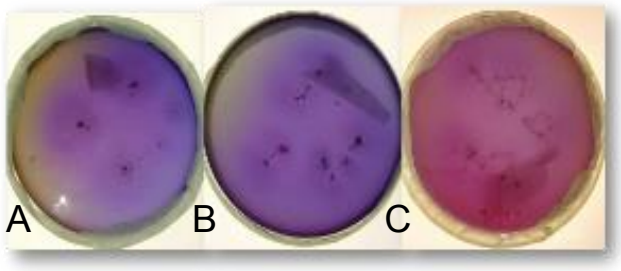

Figure 3 Chitinolytic activity of strains isolated in a medium supplemented with colloidal chitin after 72 hours. a) AM, b) NE and c) VE

4.5 Determination of crude protein and reducing sugars

Chitin is a potent inducer in the production of chitinolytic enzymes. When this carbohydrate is present in the medium as the sole source of carbon, the fungus triggers a series of metabolic processes for its growth. The concentration of reducing sugars, intra- and extracellular proteins was calculated, both at the beginning and at the end of the experimental process, to relate it to growth.

A Pearson correlation analysis with a significance level of .05 was carried out. The correlation between the extracellular proteins expressed by the fungus and the amount of reducing sugars showed a negative relationship between the variables. As more reducing sugars are expressed, the extracellular proteins may have a partial decrease in the medium.

It can be inferred that due to the low amount of N-acetylglucosamine, due to enzymatic hydrolysis, some quito-oligosaccharides were formed, such as chitobiose and chitotriosa, which could not be degraded by the fungus. In contrast, statistically the relationship between intracellular proteins was directly proportional to the final biomass concentration of the fungus. This may be an indication that proteins are enzymes that can be exported to the extracellular medium to degrade chitin. On the other hand, the data obtained from the growth of microorganisms at different pH were subjected to the analysis of variance (ANOVA), with a reliability coefficient of 95% ($p = 0.05$) to determine which treatment has greater efficiency in the production of proteins. Extracellular Statistically, isolated strains show better enzymatic activity at a pH of 4.

The above is related to biomass, since the fungi AG2, AG4, NE and AM generated higher biomass yield at pH 4. Some research shows that genera of Beauveria and other entomopathogenic fungi can grow in conditions where there are few nutrients and in a wide range of pH (Shimazu and Sato 1996).

4.6 Bioassays with insects

For the final bioassays, tests were carried out with Acheta domesticus, Germanic Blattella and Musca domestica; which, can be considered as plagues in the region. In the first place, the Acheta domesticus species (Figure 4, section A) was vulnerable to all the isolated strains. Growth was shown after 96 hours of incubation at room temperature. The fungi grew irregularly by the trunk of the insect and the Petri box. In the case of the Blatella germanica species (Figure 4, section B), a greater development of the AG1 fungus (genus Beauveria) was observed in the insect compared to the other isolates. The fungus showed a mycelial growth on the insect starting at 72 hours, covering part of the trunk and extremities.

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It is worth mentioning that it was the insect that had the greatest resistance to the growth of microorganisms. Only considerable growth was observed with strains AG1, AG4 and AG5. On the other hand, a significant affectation was observed in the species Musca domestica (Figure 4, section C) by the fungus AG5 that attacked a large part of the trunk and wings. Said growth was visible after 72 hours of incubation.

Tests were carried out on each of the 8 species of fungi that adapted to the minimum environment, mainly focused on the species Acheta domesticus, where a large general affectation was observed on the trunk of the species by the strains AG1, AG2 and AG5.

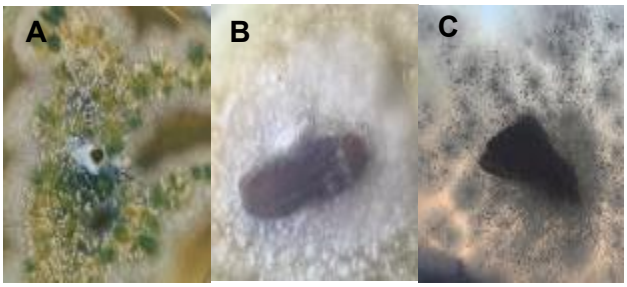

Figure 4 Bioassays performed with different insects considered as pests. A) Mushroom growth of the genus Metarhizium on Acheta domesticus at 96 h-1. B) Growth of the fungus of the genus Metarhizium on Germanic Blatella and C) Growth of the fungus AG5 on the moth

Previous studies have shown that entomopathogenic fungi, including Beauveria and Metarhizium species, produce proteases, chitinases and lipases that can degrade the cuticle of insects, favoring colonization (Charnley, 2003). The pathogenesis of the fungus on insects implies several factors for it to be carried out. The main factors are the host, the parasite and the environment; as well as, the metabolic capacity of the fungus so that it can degrade chitin. The penetration of the fungal pathogen in the cuticle depends in a relevant way on the action of the enzymatic activities (Castellanos-Moguel et al., 2008).

In this study the strains that showed the most activity on the pests were those that grew in the exoskeleton of the insect after 96 hours.

4.7 Future perspectives

There are several points that should be considered in further investigations such as: a) the selection of isolates with high chitinolitic potential for the infection of pests and the use of their biological derivatives (enzymes), b) the correct characterization of the most important fungal genera in this area of study, c) the study of the activity on pests present in crops of the region and d) the planning and implementation of technologies that can use this phenomenon of in vivo pathogenicity.

5. Conclusions

Of the 14 asylums, only 8 showed the ability to use chitin as the sole source of carbon and at the same time entomopathogenic activity on the genera Acheta domesticus, Germanic Blatella and Musca domestica, the former having the most exoskeletal involvement. There is a proportional relationship between the chitinolytic activity and the concentration of reducing sugars, presuming the presence of strong enzymatic mechanisms in strains AM, NE and VE, which served as the basis for determining the chitinolytic activity by means of the colorimetric test, taking its best yields at pH 4.

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Pteridoflora of the Área de Protección de Flora y Fauna La Primavera, Jalisco, Mexico

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Abstract

A taxonomic list of the pteridophytes of the Área de Protección de Flora y Fauna La Primavera is presented. This area is located on the west of the Zona Metropolitana de Guadalajara and comprises an area of 30,500 ha, which includes four municipalities and extends from an altitude of 1,400 to 2,200 m. The information about ferns and fern allies in the Natural Area is scarce, so the main goal of this research was to contribute to the knowledge of the richness and distribution of pteridoflora in different plant communities. The study was based on exhaustive checking literature, exicatae and specimen collection in in different types of vegetation. We found 77 species, which are grouped into 35 genera and 17 families. The most diverse genera are *Cheilanthes* (14 spp.), *Adiantum* (7 spp.) and *Thelypteris* (6 spp.). 33 species are cited for the first time for this area and *Macrothelypteris torresiana* for the state. Three species were recorded in Official Mexican Standard NOM-059-SEMARNAT-2010, *Campyloneurum phyllitidis, Nephrolepis cordifolia* and *Selaginella porphyrosphora* in the category of non-endemic and endangered species. In addition, *Psilotum nudum, Dennstaedtia bipinnata*, *Lycopodiella cernua* and *Equisetum hyemale* var. *affine* are sparce in the area.

Ferns and fern allies, Floristic checklist, Bosque La Primavera, Jalisco

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Introduction

The pteridophytes are vascular plants without flowers and they reproduce through spores instead of seeds, they differ of Bryophytes and Spermatophytes by their life cycles where sporophyte and gametophyte are independent at maturity (Martínez-Salas y Ramos, 2014).

Worldwide there are around 12,000 species of pteridophytes, concentrating up to 70-80% in the intertropical regions, while only 20% are found in the temperate regions of the globe (Salvo, 1997). They are more common in humid and slightly seasonal tropical and subtropical mountains. The greatest diversity of species is located in two large regions where about 75% of the 9,000 species are found. One of those centers of diversity is the southeast of Mexico and the other Central America (Tryon and Tryon, 1982).

In general, this group of plants has not been part of the conservation programs of the threatened floras and to decree the degree of threat of the species that inhabit certain areas it is very necessary to have an updated list of the taxonomic entities present in them (De la Sota et al., 2009). The search for biodiversity patterns without previous taxonomic knowledge can not be conceived and this supposes not only knowing in a reliable way the species that inhabit a given space, but also their distributions through that space (Toledo, 1994). Therefore, in this paper we report a pteridofloristic checklist of the Area of Protection of Flora and Fauna La Primavera (APFFLP), Jalisco.

Justification

The APFFLP is a priority area for conservation in the Zona Metropolitana de Guadalajara and in Jalisco. However, until now there is no specific publication regarding its pteridophytes.

Therefore, there is no accurate knowledge of which species live there, since the lists that exist are very general, for specific areas and / or are incomplete. Díaz-Barriga and Palacios-Ríos (1992) mention that ferns and related plants are a group of plants of great biological importance, due to their good representation in the fossil record and their high dispersion capacity. In addition, knowledge of the list of species that grow there contributes to their management and conservation.

Problem

The APFFLP is the largest natural reserve within the ZMG. This makes it a laboratory and object of several kinds of scientific research. In addition, due to its proximity to the ZMG it is constantly visited by the general public; thus, the species are affected by the increase in the disturbance of their different ecosystems. Some examples of these types of alterations are logging, fires, subdivision development, pollution or other anthropogenic factors are examples of these types of alterations. The ignorance of the pteridological richness of the area makes them vulnerable to these problems.

General objective

Know the pterido floristic diversity of the Area of Protection of Flora and Fauna La Primavera, Jalisco.

Specific objetives

- Perform the inventoryof the fern and similar plants of the APFFLP.
- Provide information about the distribution of species in the study area by vegetation types.

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Theoretical framework

Pteridofloristic diversity in Mexico is high, which has been reflected in recent works (Mickel and Smith 2004, Martínez-Salas and Ramos, 2014). In western Mexico, within the area called Nueva Galicia, according to Mickel (1992), there are 281 species of this group of plants. And specifically for the state of Jalisco there are registered 24 families, 66 genera and 259 species of pteridophytes (Ramírez-Delgadillo et al., 2010a). In addition, different floristic studies of important natural areas in Jalisco have been published, among those that report a greater number of species of the group under study are:

Vázquez and collaborators (1995), refer to the Flora de Manantlán a total of 165 taxa of pteridophytes distributed in 18 families, being the genus *Asplenium* the most diverse with 18 species. On the other hand, Cuevas-Guzmán and Jardel-Peláez (2004) reported 73 species to the Flora of the Scientific Station Las Joyas. In Flora of Northern Jalisco, Vázquez et al. (2004) reported 74 taxa of pteridophytes, which represent 3.55% of a total of 2081 species inventoried in the region. Machuca-Sánchez (1986) records 40 taxa for the northern region of the municipality of Jocotepec. Cortés-Romero (2000) considers 35 species for the Cajititlán region in the municipality of Tlajomulco de Zúñiga, Jalisco. And in El Tepopote hill, Jalisco Mexico, Frías-Castro et al. (2013) indicate the presence of 31 species of pteridophytes.

Regarding the floristic character works that have been carried out in the La Primavera Forest (BLP) and nearby areas, we can mention the following: Rodríguez-Pastrana (1953) cites 59 species for the surroundings of Guadalajara, the Management Plan of the BLP reports 21 species (Anónimo, 1988); later in a more specific area (Bosque-Escuela CUCEI, in Tala, Jalisco) Rodríguez and Reynoso-Dueñas (1992) list 11 species.

On the other hand, SEMARNAP (2000) registered 16 species in the Management Program of the ANFFLP; In another area of BLP, called El Colli hill, the authors Macías-Rodríguez and Ramírez-Delgadillo (2001) contemplate seven species. Finally, Cedano-Maldonado et al., In 2006 report nine wild species of pteridophytes useful for the metropolitan area of Guadalajara.

Research Methodology

Study area

The area called APFFLP has an area of 30,500 ha, its geographical location corresponds to the coordinates: 20 ° 37 'and 20 ° 45' latitude N and -103 ° 35 '-103 ° 28' longitude O belongs to four municipalities, here mencioned by order, and the percentage is refered to the extension the protected area occupies within them: Zapopan (48%), Tala (37%), Tlajomulco (12.5%) and Arenal (2.5%) (Figure 1).

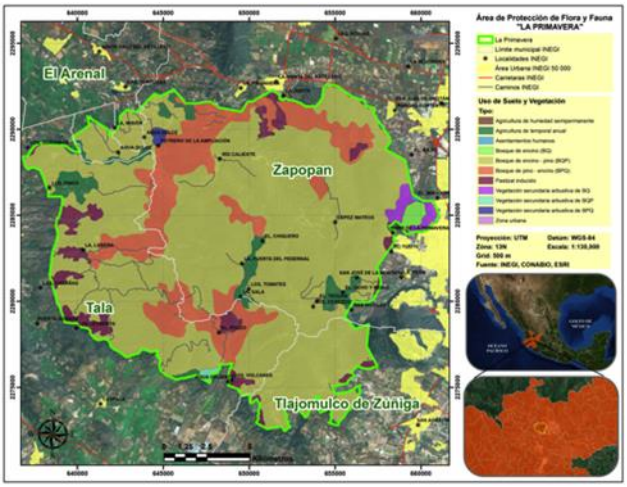

Figure 1 Location of the study area

It is located in a set of five valleys (Tala, Tesistán, Toluquilla, Atemajac y San Isidro Mazatepec) (Anónimo, 1988). In the APFFLP, the physiographic variation is manifested in an altitudinal range of 1400-2200 m and comprises the overlap zone between two floristic provices:

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The Sierra Madre Occidental and The Sierras Meridionales or Eje Neovolcánico Transversal, and is made up of mountainous areas, where different plant communities are found. According to Reyna (2004), in La Primavera there are five types of vegetation: pine forest, oak forest, tropical deciduous forest, riparian vegetation and secondary vegetation or altered sites. In the area, approximately 1000 species of plants are distributed (SEMARNAP, 2000).

Materil and methods

Specialized literature for the study area was reviewed, and the specimens corresponding to collections of pteridophytes made within the APFFLP of the herbariums of the Institute of Botany "Luz María Villarreal de Puga" of the University of Guadalajara (IBUG) and of the Faculty of Biology "Carlos Luis Díaz Luna" of the Autonomous University of Guadalajara (GUADA) were checked exhaustively.

For this study, it was collected in 49 localities with easy access in terms of roads, throughout the APFFLP. Preferably in those humid places such as streams and ponds. The explorations were developed from 2011 to 2013, with some sporadic outings in 2014.

The herborization was done in the traditional way for this group of plants and its determination was based on Tryon and Tryon (1982), Mickel and Beitel (1988), Mickel (1992) and Mickel and Smith (2004). The first set of vouchers was deposited in the herbarium IBUG, other collections to which the duplicates were distributed include MEXU, ZEA, IEB, ENCB, HUAA, XAL and CIDIIR.

Results

Through the review of 215 records in the IBUG herbarium and 119 records in GUADA, as well as 415 collection numbers, the presence of 77 species of pteridophytes was determined in the APFFLP, which are grouped into two divisions, six orders, 18 families and 35 genera (Appendix 1).

The most representative families in terms of number of species are Pteridaceae, which contributes the most, with 42%, Polypodiaceae with 10%, Thelypteridaceae with 9% and the 5% for families Aspleniaceae and Schizaceae. In Table 1 the families of the species included in this work are cataloged. In addition, the number of genera and species grouped is shown.

Family	No. of genera / Species
Aspleniaceae	¼
Athyriaceae	3/3
Azollaceae	1/1
Blechnaceae	2/2
Dennstaedtiaceae	2/2
Dryopteridaceae	3/5
Equisetaceae	1/1
Lycopodiaceae	1/1
Marsileaceae	1/1
Ophioglossaceae	½
Osmundaceae	1/1
Polypodiaceae	4/8
Psilotaceae	1/1
Pteridaceae	8/32
Schizaceae	¼
Selaginellaceae	½
Thelypteridaceae	2/7

Table 1 Families of pteridophytes present in the APFFLP and their number of genera and species. (arranged alphabetically).

The most diverse genera are Cheilanthes with 14 species, Adiantum (7), Thelypteris (6) and Polypodium (5) (Graph 1).

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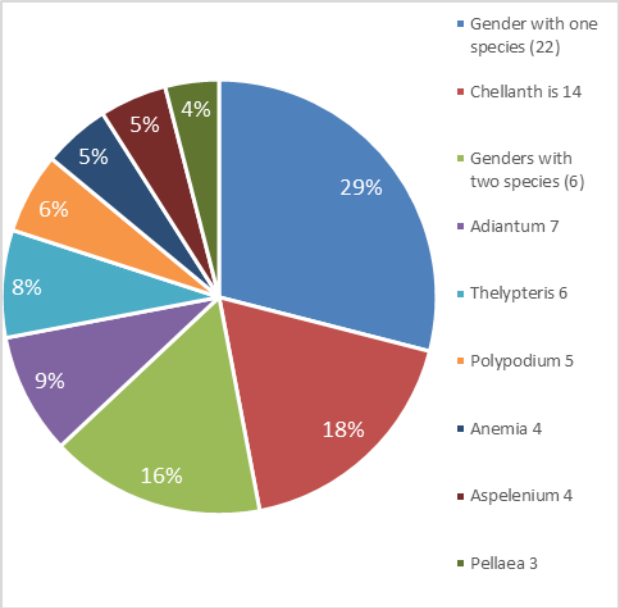

Graph 1 Genres with the highest number of species and their percentage with respect to the total species of this study.

Compared with Mickel & Smith (2004), 58% of the families, 28% of the genera and 7% of the species mentioned for Mexico are present in the study area. In addition, 17 of the 25 families were found, 34 of the 65 genera and 77 of the 265 species of ferns and allies that Ramírez-Delgadillo et al. (2010) reported for Jalisco.

Among the genera with greater richness we have *Cheilanthes*, *Adiantum* and *Thelypteris*, this is similar to other floristic works such as those of Vázquez-García et al. (1995), Hernández-Toro (2003) and Vázquez-García et al. (2004).

Rodríguez-Pastrana (1953) mentions 17 species for the area of La Primavera. But while the revision of herbarium was made, it was detected that its collections contribute to the present list with nine more species. These were included in his thesis, but without detailing his presence in the area.

And also two other species are added by the specimens *Rodríguez 104* and *Rodríguez 61*, *Thelypteris cheilanthoides* var. *cheilanthoides* and *Thelypteris pilosa* respectively, but these are absent in his work. On the other hand, it was determined that *Rodriguez 19* previously identified as *Pteridium caudatum* is *Pteridium aquilinum* var. *feei*. Another species cited in his study that is presumed to be misidentified was *Dryopteris patula* (*17* and *25 August 1952*, specimens not seen) and probably *Dryopteris rossi*. Finally, the species that were not found during the field trips were *Bommeria hispida*, *Azolla microphylla* [= *Azolla mexicana*] and *Tectaria trifoliata*, the last two are cited in their work, but no specimens were found.

Anónimo (1988) lists the species *Woodwardia radicans* and *Dryopteris mexicana* which were not found in field, and since that work does not mention exsiccatae, it is not possible to know exactly what these species are, but it is probable that they are *W. spinulosa* and *D. rossi.*

Mickel (1992) presents 37 species and a total of 39 records for the study area. Of these, the species that were not found in the botanical explorations carried out were *Asplenium hallbergii*, *Campyloneuron phyllitidis*, *Mildella fallax* [= *Mildella intramarginalis var. serratifolia*], *Ophioglossum reticulatum* and *Thelypteris cheilanthoides* var. *cheilanthoides*.

The specimen *Rodriguez August 17, 1952* previously identified as *Diplazium lonchophyllum* is about *Thelypteris pilosa*. And the specimen *Puga 7099*, cited in the same work as *Psilotum complanatum*, is a homonymous locality (Arroyo de Agua Caliente) and the specimen is identified by the own Mickel in 1990 as *P. nudum*.

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Otherwise, in SEMARNAP (2000), *Blechnum occidentale* and *Selaginella lepidophylla* are mentioned. These species were not found in the area so they could have been misidentified. Since there are no exsiccatae, it is believed that they could correspond to *B. appendiculatum* and *S. pallescens*.

In this study *Macrothelypteris torresiana* is cited to the state for the first time, this species is invasive and is increasing its distribution area within Mexico (Mickel & Smith, 2004) and in the study area it was observed in disturbed areas; in addition, the following species are new records for the APFFLP: *Adiantum andicola, A. tricholepis, Anemia tomentosa* var. *mexicana, Asplenium monanthes, A. pringlei, A. pumilum, Astrolepis sinuata, Athyrium palmense, Bommeria hispida, Cheilanthes allosuroides, C. aurantiaca, C. bonariensis, C. brachypus, C. chaerophylla, C. cucullans, C. lozanoi* var. *seemannii, C. membranacea, C. skinneri, Cheiloplecton rigidum* var. *rigidum, Cystopteris fragilis, Dennstaedtia bipinnata, Dryopteris maxonii, Nephrolepis cordifolia, Ophioglossum crotalophoroides, Osmunda regalis* var. *spectabilis, Pellaea ovata, Pityrogramma calomelanos, Pleopeltis mexicana, Selaginella pallescens, Thelypteris hispidula, Thelypteris resinifera* var. *resinifera* and *Thelypteris oligocarpa*.

The species that have a protection category in the Official Mexican Standard NOM-059-SEMARNAT-2010 (non-endemic and endangered) are *Nephrolepis cordifolia* and *Selaginella porphyrosphora*. In addition, *Psilotum nudum, Dennstaedtia bipinnata, Lycopodiella cernua* and *Equisetum hyemale* var. *affine* are scarce in the APFFLP. No endemic species was found to the state of Jalisco.

Conclusions

With 77 species found, the APFFLP presents a high richness of fern and allied species in Jalisco. Many of them detected only through herbarium specimens. Therefore, it is suggested to implement conservation and restoration actions in order to prevent the loss and maintain the number of species. As it is appreciated, the area is an important habitat for this group of plants. Figure 3 shows some of the main species registered.

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Figure 3 *Diversity of pteridophytes present in different APFFLP habitats:* **a)** *Selaginella pallescens in oak forest;* **b)** *Thelypteris pilosa in gallery forest with oak forest;* **c)** *Anogramma leptophylla in oak forest;* **d)** *Adiantum patens in tropical deciduous forest;* **e)** *Cheilantes brachypus in oak forest;* ***f)*** *Asplenium pringlei in tropical deciduous forest;* **g)** *Cheilanthes myriophylla in tropical deciduous forest;* **h)** *Dryopteris rossii in tropical deciduous forest;* **i)** *Elaphoglossum muelleri in oak forest;* **j)** *Phlebodium areolatum in deciduous tropical forest;* **k)** *Polypodium thyssanolepis in tropical deciduous forest and* **l)** *Psilotum nudum in gallery forest with oak forest.*

. Cols. = Collectors; AAR = A. Alvarado C .; AR = Amancio Rodríguez; ARR = Antonio Rodríguez Rivas; AS = Aura Star; CLDL = Carlos Luis Díaz Luna; DG = David García), DLG = Dora Leticia González; EC = Emilio Camarena; EE = Eduardo Espinoza; EPA = Ernesto Pérez Arce; FJMN = Francisco Javier Muñoz Nolasco; FML = Fabián Marrero León; GPR = Gabriel Pérez Rulfo; JAG = Jaime A. García; JPL = Jesús Padilla Lepe; LFCN = Luis Fernando Colin Nolasco; LHL = Leticia Hernández López; LL = Laura Leopo; LMVP = Luz María Villarreal de Puga; MAMR = M. A. Mejía Rodríguez; MEC = Martha E. Castrejón; ORB = Oscar Reyna Bustos and PESH = Pedro E. Sánchez Hernández. Habitat: A = Aquatic; E = Epiphyte; T = Terrestrial and R = Rupicola. Type of Vegetation: BE = Encino Forest; BEP = Encini-Pine Forest; BG = Gallery Forest; BP = Pine Forest; BPE = Pine-Encino Forest; Deciduous Tropical Forest; VA = Aquatic Vegetation and VS = Secondary Vegetation.

	Species	**Collections./Num collection**	**Habitat**	**Type of vegetation**	**No. Records**
	LYCOPHYTA				
	Lycopodiaceae				
1	*Lycopodiella cernua (L.) Pic. Be M.*	JQ 10 febrero 1976, LFCN & EC 450	T	VS c/ elem. BPE	4
	Selaginellaceae				
2	*Selaginella pallescens (C. Presl) Spring*	EE 5447, LFCN *et al.* 772	T, R	BPE, BG, VS, BEP, BE, BTC	34
3	*Selaginella aff. porphyrospora Mickel & Beitel*	DLG 6, IH 1041	T	BPE	2
	MONILOPHYTA				
	Aspleniaceae				
4	*Asplenium hallbergii Mickel & Beitel*	Acosta 17783	T	BPE	1
5	*Asplenium monanthes L.*	AR 66, LFCN & LHL 1101	T	BPE c/elem. BTC, BE	6
6	*Asplenium pringlei Davenp.*	LFCN *et al.* 536, LFCN & FJMN 765	T	BTC, BG c/elem. BTC	2
7	*Asplenium pumilum Sw.*	EPA 17010, LFCN 421	T	BG, BPE	2
	Athyriaceae				
8	*Athyrium palmense (Christ) Lellinger*	LFCN *et al.* 205, LFCN & ARR 961	T, E	BG	2
9	*Cystopteris fragilis (L.) Bernh*	LHL *et al.* 1038, LFCN *et al.* 441	T	BG, BPE, BEP	4
10	*Woodsia mollis (Kaulf.) J. Sm.*	JAG 16, LFCN *et al.* 436	T	BEP, BPE, BG, BE, BTC, VS	16
	Azollaceae				
11	*Azolla Mexican Presl.*		A	BG	1
	Blechnaceae				
12	*Blechnum appendiculatum Willd.*	LMVP 14849, LFCN *et al.* 565	T	BG, BPE, BE	15
13	*Woodwardia spinulosa M. Martens & Galeottii*	AR 26, LFCN *et al.* 586	T	BPE, BG, VS	29
	Dennstaedtiaceae				
14	*Bipinnate dennstaedtia (Cav.) Maxon*	LFCN 680	T	BG	2
15	*Pteridium aquilinum var. feei (W. Schaffn ex Fée) Maxon ex Yunck.*	AR 19, LFCN *et al.* 678	T	BPE, BG, VS, BEP, BE, BP	41
	Dryopteridaceae				
16	*Dryopteris maxonii Underw. & Chr.*	LL 25 agosto 1985, LFCN 549	T	BG, BPE, BEP, BTC, BE, VS	13
17	*Dryopteris rossii C. Chr.*	LMVP 3116, LFCN *et al.* 577	T	BPE, BEP, BG, VS, BP, BE, BTC	46

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18	*Elaphoglossum muelleri (E. Fourn.) C. Chr.*	DG 4959, LFCN *et al.* 587	T	BPE	8
19	*Nephrolepis cordifolia (L.) C. Presl*	LFCN *et al.* 125	T	BG	1
20	*Nephrolepis undulata (Afzel, Ex Sw.) J. Sm.*	AR 53, LFCN 558	T	BG, BEP, BE, VS	11
	Equisetaceae				
21	*Equisetum hyemale var. affine (Engelm.) A. A. Eaton*	CLDL 853, LFCN 422	T	BG c/ elem. BPE	8
	Marsileaceae				
22	*Marsilea mollis B. L. Rob. & Fernald*	LMVP 5692, LFCN *et al.* 975	A	VA	7
	Ophioglossaceae				
23	*Ophioglossum crotalophoroides Walter*	LFCN 250, 439	T	BTC, BEP	2
24	*Ophioglossum reticulatum L.*	LMVP 599	T	BPE	4
	Osmundaceae				
25	*Osmunda regalis var. spectabilis (Willd.) A. Gray*	MAMR 20 enero 1982	T	Áreas inundadas y BPE en Mickel (1992).	1
	Polypodiaceae				
26	*Campyloneurum phyllitidis (L.) C. Presl*	DG 4957	E, R	BE	2
27	*Phlebodium areolatum (Humb. & Bonpl. Ex Willd.) J. Sm.*	AR 18, LFCN *et al.* 560	E, R	BPE, BEP, BG, BTC, VS, BE	20
28	*Pleopeltis mexicana (Fée) Mickel & Beitel*	LFCN &JPL 958	E	BG	1
29	*Polypodium furfuraceum Schlecht. & Cham.*	DG 15, LFCN *et al.* 569	E	BPE, BG, BE	8
30	*Polypodium madrense J. Sm.*	LFCN 338, 438, 679, 1100	E	BPE, BEP, BG	4
31	*Polypodium plesiosorum Kunze*	LMVP 3132, LFCN *et al.* 537	T, R	BPE, BEP, BG, BTC, VS	15
32	*Polypodium polypodioides var. aciculare Weath.*	FML 24 agosto 1985, LFCN & JPL 957	E	BG, BPE	3
33	*Polypodium thyssanolepis A. Braun ex Klotzsch*	AR 29, LFCN & JPL 366	T, E	BTC	5
	Psilotaceae				
34	*Psilotum nudum*	LMVP 9503, LFCN 575	R, E	BG c/ elem. BPE y BE	4
	Pteridaceae				
35	*Adiantum andicola Liebm.*	AR 110, 201 C	T	BPE	2
36	*Adiantum braunii Mett. ex Kuhn*	AR 63, LFCN *et al.* 584	T	BG, BE, BEP, BPE, BTC.	21
37	*Adiantum capillus-veneris L.*	LFCN 572	T	BG c/elem. BPE	2
38	*Adiantum concinnum Humb. & Bonpl. ex Willd.*	AR 48, LFCN *et al.* 576	T	BPE, BG, BEP, BE	34
39	*Adiantum patens Willd.*	LMVP 3117, LFCN & JPL 253	T	BG, BPE, VS	13
40	*Adiantum poiretii Wikstr.*	LMVP 2448, LFCN *et al.* 675	T	BPE, BG, BP, VS	19
41	*Adiantum tricholepis Fée*	AR 17 agosto 1952	T		1
42	*Anogramma leptophylla (L.) Link*	LFCN 442, 952	T, R	BPE	3
43	*Astrolepis sinuata (Lag. Ex Sw.) D.M. Benham & Windham*	DG 4956, LFCN *et al.* 312	T	BPE	2
44	*Bomp hispida (Mett. Ex Kuhn) Underw.*	AR 38	T	BPE, BTC	1
45	*Bomma pedata (Sw.) E. Fourn*	LMVP 4604, LFCN 574	T, R	BPE, BG, BEP, BTC, VS	32
46	*Cheilanthes allosuroides Mett.*	LFCN & JPL 373	T	BTC, BG c/elem. BTC	4
47	*Cheilanthes aurantiaca (Cav.) Moore*	LMVP 4076, LFCN el al. 582	T	BPE	4
48	*Cheilanthes angustifolia Kunth*	LMVP 1609, LFCN *et al.* 539	T, E	BPE, BG, BEP, VS	44
49	*Cheilanthes bonariensis (Willd.) Proctor*	LMVP 1968, LFCN *et al.* 678	T	BPE, BEP, BE, BTC	14
50	*Cheilanthes brachypus (Kunze) Kunze*	LMVP 4094, LFCN *et al.* 581	T, R	BPE, BG, VS, BEP	27
51	*Cheilanthes chaerophylla (M. Martens & Galeotti) Kunze*	PESH 18 julio 1997, LFCN 171	T	BG, BE	5
52	*Cheilanthes cucullans Fée*	LFCN & ARR 20		BG c/elem. BTC y BE	1
53	*Cheilanthes farinosa (Forssk.) Kaulf.*	EE 5454, LFCN 316	T, R	BPE, BEP	4
54	*Cheilanthes kaulfussii Kunze*	DG 27 julio 1969, LFCN *et al.* 676	T	BPE, VS, BG, BEP, BP, BE, BTC	52
55	*Cheilanthes lozanoi var. seemannii (Hook.) Mickel & Beitel*	DG 27 julio 1969	T	BPE	2
56	*Cheilanthes membranacea (Davenp.) Maxon*	MEC 18 octubre 1979	T	BPE	1
57	*Cheilanthes myriophylla Dev.*	AR 27, LFCN & JPL 371	T	BTC, BPE	6
58	*Cheilanthes pyramidalis Fée*	AR 22, LFCN *et al.* 959	T	BPE, BEP, BE, VS	21
59	*Cheilanthes skinneri (Hook.) T. Moore*	DG 27 agosto 1969	T	BPE	1
60	*Cheiloplecton rigidum. var. rigidum (Sw.) Fée*	CSC 27 septiembre 1986, LFCN *et al.* 771	R, T	BEP, BPE, BE, VS	5
61	*Mildella falax (M. Martens & Galeotti) G. L. Nesom*	CLDL 5575	T	BPE	2
62	*Pellaea cordifolia (Sessé & Moc.) A. R. Sm.*	ORB & AAR 676	T	BPE	1
63	*Pellaea ovata (Dev.) Weath.*	DG 55, LFCN 955	T	BPE	3
64	*Pellaea ternifolia (Cav.) Link*	AR 13, LFCN *et al.* 579	T	BPE, BEP, BE, BG, BTC	13
65	*Pityrogramma calomelanos (L.) Link*	AE 29 marzo 1972, LFCN *et al.* 28	T	BG	3
66	*Pityrogramma ebenea (L.) Proctor*	LMVP 4099, LFCN *et al.* 677		BPE, BG, VS, BE, BP	29
	Schizaceae				
67	*Anemia hirsuta (L.) Sw.*	GPR 17036, LFCN *et al.* 169	T	BG, BPE	4
68	*Anemia jaliscana Maxon*	LMVP 2105, LFCN & EC 451	T	VS, BPE, BTC, BG	6

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69	*Anemia karwinskiana (C. Presl) Prantl*	ECG 11113, LFCN *et al.* 535	T	BPE, BE, BG, BTC	8
70	*Anemia tomentosa var. mexican*	DG 24	T	BPE	1
	Thelypteridaceae				
71	*Macrothelypteris torresiana (Gaudich.) Ching*	LHL *et al.* 1040, LFCN *et al.* 552	T	BG, BEP, BPE, VS	11
72	*Thelypteris cheilanthoides var. cheilanthoides*	AR 104 (19 agosto 1952)	T	BPE (según Mickel, 1992)	2
73	*Thelypteris hispidula (Decne.) C.F. Reed*	LFCN 570	T	BG c/ elem. BPE	9
74	*Thelypteris pilosa (M. Martens & Galeotti) Crawford*	AR 61 (17 agosto 1952), LFCN 571	T	BG c/ elem. BPE	10
75	*Thelypteris resinifera var. resinifera*	DG 5389, LFCN *et al.* 566	T	BG c/ elem. BPE	18
76	*Thelypteris oligocarpa (Willd.) Ching.*	IH 1042	T	BPE	1
77	*Thelypteris puberula var. sonorensis A. R. Sm.*	ARC & JJRD 1121	T	BG	2

Table 2 Pteridoflora of the Flora and Fauna Protection Area La Primavera, Jalisco, Mexico. The list is presented in alphabetical order by family, gender and species and is divided into the following groups Lycophyta and Monilophyta

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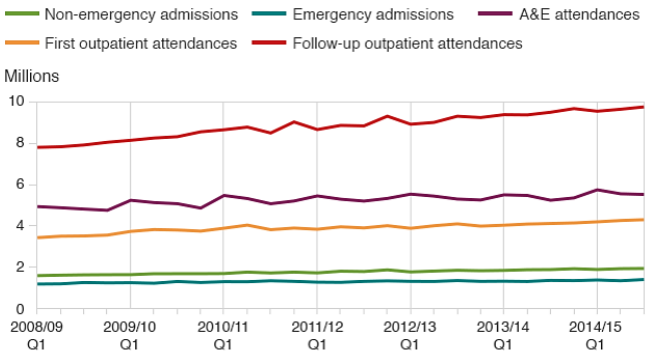

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