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Use of Medical Plant Extracts for *in Vitro* Control of *Fusarium* sp.

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Abstract **– Since Green Revolution, pest management programs have been focused almost exclusively in chemical pesticides. In particular, for the plant pathogens fungi combat, it has been vital the use of chemical fungicides, and has been stimulated by the pest resistance development and invariably by the increase concern about producers and consumers health as well as the environment. Furthermore, the increase in production cost, chemical pesticides efficiency and a further demand of safe agricultural products, it has augmented the exploration of natural alternatives for plant pathogens control. Within these strategies, the study of medicinal plants and their extracts for use as natural control of plant pathogens promises be a powerful tool in sustainable pest management. In this sense, exist plenty of information that support the efficacy of plants to inhibit the fungi growth. *Fusarium* spp., cosmopolite plant pathogen fungi, due their big severity in host plants and resistance to commercial pesticides, have become in focus of a lot investigation oriented in his control. This work presents a small overview about the study of plants in México for control of *Fusarium*sp, and the evaluation of three medicinal plant extracts as *in vitro* radial growth inhibitors of local *Fusarium* sp. fungi. It was found susceptibility of *Fusarium* sp. to plant extracts of Siete Colores (*Lantana hirta*), Chicalote (*Argemone ochroleuca*) and Árnica de Cerro (*Adenophyllum porophyllum*). The ethanol extract of Siete Colores and Chicalote show 100 % of inhibition, coupled with 80 % of inhibition by Árnica de Cerro, proved that the use of these plants can be a viable option to explore in the sustainable management of plant pathogen fungi.**

Keywords **– Argemone Ochroleuca, Adenophyllum Porophyllum, Lantana Hirta, Plant Pathogen.**

I. INTRODUCTION

Fusarium spp. are between the most important plant pathogens distributed in a big diversity of environments and spread in all the world [1], there have the capacity of infection and cause plant disease in different crops as vascular wilts, leaf spots, blights and decay of roots, stems, leaves, fruits, grains, and seeds [2]. At present, still are looking options for the control of these plant pathogens, principally by the increase in efficacy of pesticides and the preference intensification of agro ecological management of crops [3]. Among the most promising alternatives are the integrated pest management that includes, cultural, biological and natural pest control. Of the above, it was strengthened the exploration of plant extracts for pest control. Several studies support that the plant extracts can be effective against mites, rodents, nematodes, bacteria, virus, fungi and insects [4]-[5]; also their not present harmful to soil and environmental effects such as chemicals contained in commercial fungicides. The success of pest control programs not only imply the knowledge of the crop, pest and their control, but also, must be supported by the understanding of the agro ecological components for a better improvement of local biotic resources. The information of pesticides capacity of local wild plants could be the first step for the development of a sustainable pest management strategies accessible for local farmers. The objective of this work is present the results of the evaluation of inhibitory effects of three extracts of local medical plants over *in vitro* mycelia radial growth of *Fusarium* sp.

Fusarium sp. in México.

The members of genus *Fusarium* are naturally in soils, and can be associated with rots roots and stems of many plants. The *Fusarium* species are saprophyte in some of their growth faces, and can or cannot develop a sexual reproduction phase by specie [6]. They are cosmopolite organism with some endemic species [7]. In México *Fusarium* spp. are one of the most economical important plant pathogens, because their can colonize organs of some of the most important cultivated plants as corn (*Zea mays*), bean (*Phaseolus vulgaris*) y asparragus (*Asparagus officinalis*), between others; also are very difficult to control [6]. In these sense it was reported the resistance of *Fusarium* to synthetic fungicides as acibenzolar S-methyl, carbendazim, azoxystrobin and tebuconazole [8]. Foregoing has stimulated the looking of natural alternatives for pest management provoked by this fungi.

Use of Plant Extracts for Control of Plant Pathogens Fungi in México.

Estimates made in the 90´s decade show that the diversity of higher plants in México are between 23 and 30,000 species [9]; however, the knowledge about their chemical diversity is actually limited and remaining studding the structure of thousands of compounds of chemical profile of each plant. The presence of this compounds respond to their ecological situations, and form the complex response and defense mechanism developed a long their evolution. Under these compounds, highlights the secondary metabolites with anti-microbial properties [5]. The secondary metabolites are present as compounds mixtures called crude extracts [10], and they are shown fungicides effects against diversity of plant pathogens as *Alternaria* sp., *Colletotrichum* sp., *Botrytis* sp., *Aspergillus* sp., *Phytophthora* sp., *Rhizoctonia* sp., *Rhizopus*sp. and *Fusarium* sp. [5]. With respect to *Fusarium* spp., in México exist reports about the antifungal effect of different plant extracts (Table I).

Table I. Somme plants used in inhibition studies of *Fusarium* spp. in México[3]-[11]-[12]-[13]-[14]-[15].

Scientific Name	Common Name
D. discolor	Manzano del desierto
A. ambrosioides	Chicura
F. cernua	Hojasén
C. zeylanicum	Canela
S. aromaticum	Clavo
N. glauca	Tabaquillo
S. molle	Pirul
A. cherimola	Chirimoya
M. indica	Mango
P. lentiscus	Lentisco
K. humboldtiana	Tullidora
C. album	Cenizo
B. vulgaris	Betabel
C. graveolens	Ruda
S. cynanchoides	Hierba trepadora
E. globulus	Eucalipto
A. farnesiana	Huizache
J. cinerea	Torito
S. macrodonthus	Quelite
B. glutinosa	Jarilla
S. rostratum	Hierba del sapo
P. parviflora	Doble garra
L. tridentata	Gobernadora
A. sativum	Ajo
A. confertiflora	Chíchibo
C. ambrosioides	Epazote
C. berlandieri	Huauzontle
C. plicata	Alejandría

In concerning to our medicinal plants, there are only some information of secondary metabolites content of *Argemone ochroleuca* (Chicalote) (for a good review about uses and composition of this medicinal plant see [11]). In case of *Lantana hirta* (Siete Colores) there are reports that indicate as component of the diet in Maya culture [12],until our knowledge there is any report about their antimicrobial activity, however a similar specie called *L.camara* have been extensively studied as medicinal, antimicrobial and anti-mutagenic, by their triterpene, among others metabolites content [13]. Al least, *Adenophyllum porophyllum* = *Dyssodia porophylla* (Árnica de Cerro), there is a further evidence about medicinal, nutritional and veterinary use, and about their presence in México [14].

II. Materials and Methods

With the propose of exploring a new usage of medicinal plants of regional distribution in La Ciénega de Chapala from Michoacán de Ocampo State, México, a collect of medicinal plants was realized from September 2014 to September 2015, and probed the effectiveness of three plant extracts on radial growth of a local strain of *Fusarium* sp. All test were realized in the Cellular Biology and Molecular Biology laboratories from University of La Ciénega Michoacán de Ocampo State (UCEM). Previously, the identification of fungi was realized [15], which come from Marcos Castellanos, Michoacán municipality in the 20°03' N and 103°00' W. The plants evaluated were: *L. hirta* (Siete Colores), *A. ochroleuca* (Chicalote) and *A. porophyllum* (Árnica de Cerro), and were collected in Sahuayo, Michoacán, México (20°01' N and 102°45' W). The vegetal material was transported in cooler for its conservation to the University installations, where it was dehydrated and pulverized to obtain the crude extract by the macerate method[16]. 15 g of fine powder of each plant was mixed with 65 ml of absolute ethanol (J.T. Baker). This extracts were maintained in darkness, to environmental temperature (25 ± 2°C) and constant agitation by 96 h. After this time, the dissolvent of each extract was evaporated in a vapor rotation system (Dragon Lab, RE 700-Pro) to 65 °C, and the total weight was obtained. Finally, the extracts were dissolved in absolute ethanol to final concentration of 10 mg/ml. Five concentrations were evaluated *in vitro* in ADS (Agar Dextrose Sabouraud) medium (0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, 5 mg/ml, 10 mg/ml), with three repetitions, plus a control (ADS medium plus ethanol). The radial growth was obtained with a manual Vernier each 24h, until the control completed its growth [17]. The mean and standard deviation of three experiments were determined. The inhibition percentage was calculated with the formula of Vincent [18], and the LC_{50} by an adjustment of exponential tendency line.

III. Results and Discussion

Extracts Effect Against Radial Growth of Fusarium sp.

The medicinal plant extracts evaluation against *Fusarium* sp. shown that the three plant species presented inhibitory effects over *in vitro* radial growth of fung iat seven days of treatment in ADS medium, and this inhibition was proportional to extract concentration (Fig.1).These findings can explains the plant content of metabolites that confers them properties as medicinal and microbial characteristics and the costume of use of these plants by Mexican people against infections. Furthermore, this results show the potential of extracts tested as an alternative to chemical fungicides in the control of fungal diseases.

Many others works have reported the inhibitory effects of plant extracts against *in vitro* growth of *Fusarium* spp. like *Cinnamomum zeylanicum*, *Annona cherimola*, *Syzygium aromaticum*, *Curcuma longa*, *Baccharis glutinosa*, *Datura discolor*, *Larrea tridentata*, *Acacia farnesiana*, *Bursera graveolens*, *Crescentia cujete*, *Momordica charantia* and *Helianthus annuus*[3]- [20]- [21]- [22]- [23]- [24]-[25], with inhibitory percent's between 50 and 100%, similar rates as reported in this work. Also, reports of the inhibitory effects of ethanol extracts against plant pathogen fungi *in vivo*, shown high susceptibility at these at0.5 mg/ml dosage [26].

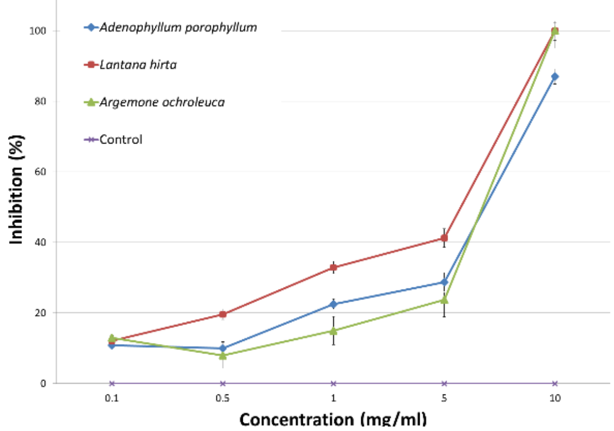

Fig. 1. Percent of inhibition of *in vitro* radial growth of *Fusarium* sp. in response to three plant extracts. Average data ± E.D. at seven days of fungi inoculation.

Respecting to their antifungal properties, ethanolic extracts of *L. hirta* have been reported as inhibitors of *in vitro* mycelia growth of the plant pathogen fungi *Colletotrichum gloeosporioides* with percent's of inhibition higher than 50% at 5 mg/ml dosage [19]. Compared with our results, this percent of inhibitions is similar to calculated for lethal dose (LC_{50}) of three extracts probed in this study, *A. ochroleuca* 4.71 mg/ml (R^2=0.719), *A. porophyllum* 4.50 mg/ml (R^2=0.8854) and *L. hirta* 3.91 mg/ml (R^2=0.9682).These results are very important because can route new explorations of use of these medicinal plants over other plant pathogens.

The treatments of ethanol extracts of *L. hirta*and *A. ochroleuca* were the most efficient in the inhibition of mycelia growth, reaching a 100% of inhibition at 10 mg/ml dosage. These inhibition kept up inclusively after 10 days of fungi inoculation (time that control trait reach and cover all the surface of the culture) (Fig. 2), more time than other commercial chemicals treatments and enough time to germination and seedlings emergence in field for treated seeds.

Particularly *A. ochroleuca* was previously reported to have antimicrobial activity in México [27], but only one work support the evaluation of Chicalote latex over *in vitro* growth of *F. oxysporum* without any antifungal effect [28]. Until our knowledge it had not reported the antifungal effects of *L. hirta*and *A. porophyllum* over *Fusarium* sp.

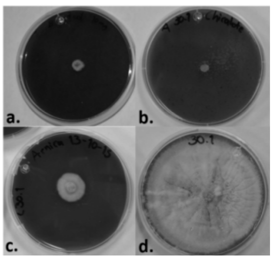

Fig. 2. Radial growth of *Fusarium* sp. Inhibition with 10 mg/ml of each extract in ADS medium. a. *L. hirta.* b. *A. ochroleuca* and c. *A. porophyllum*. d. Control, ADS medium without extract.

IV. Conclusion

Our results allow us to conclude that the improvement of local knowledge about the folkloric use of wild plants, can permit propose new alternatives of plant pathogen fungi management. The medical plants Siete Colores (*L. hirta*), shown a higher inhibitory capacity (100%) again *in vitro* radial growth of *Fusarium* sp. at 10 mg/ml dosage, in addition to presenting the lowest value in LC_{50}, which places it as the best plant species to consider study of inhibition of spore germination, and other *in situ* studies of susceptibility of fungi in cultivated plants in México.

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