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Morphological identification of phytopathogenic fungi in the guanajuatense shallow

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Abstract

This research was conducted in the guanajuatense shallows during 2014-2016 and consisted of plant pathogenic fungi isolate and analyze samples from 254 crops (tomatoes, peppers, onion, cucumber, broccoli, lettuce, carrots, strawberries, raspberries, spinach, kale and horseradish) and of germinating trays and irrigation water. The identification of microorganisms is performed through the morphological characteristics of the colonies, as well as reproductive structures of pathogens, which depending on their nutritional requirements were placed in various culture media for identification, thereby were determined the causal agents of most common diseases that occur in the guanajuatense shallows. One of the contributions sought for this study was encouraging producers to implement preventive and control strategies to reduce the damage caused by these pathogens. 107 samples were analyzed from cultures (plants), 63 soil, seed 75, six of germinating trays and three irrigation water. A total of 275 pathogens were obtained in 130 samples grouped into 10 genera and 14 species. Strawberry three main pathogens were *Rhizoctonia solani*, *Verticillium albo-atrum* and *Fusarium oxysporum* were presented. Tomato was identified *Alternaria solani* and *Phytophthora* sp. According to the results, the fungus *Rhizoctonia solani* was the pathogen most frequently found in both plants and seed and soil samples. In the germinating trays it was primarily identified *Fusarium oxysporum* and irrigation water to *Pythium* sp and *Trichoderma* sp. The rest of the samples tested negative for fungi.

Fungi, phytosanitary diagnosis, vegetables

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Introduction

The importance of fungi in nature can hardly be overestimated, essentially because more than 100,000 species are known, most of which are saprophytes; However, about 50 species produce diseases to man and approximately 8,000 directly affect plants, having the capacity to attack and infect wild and cultivated species, causing damages that can be slight until the crops are lost in their totality (However, However, damage caused by phytopathogenic fungi is not only economical, but also disrupts ecosystems, limiting cultivation areas rendering them unusable by the formation of survival structures such as sclerotia and chlamydospores that ensure their permanence in the soil for long periods of time. Time and by their ability to produce pectolytic enzymes that degrade the cells' mean lamella (Harman and Shores, 2007). Infected plants suffer a series of alterations, which can affect the morphology or external appearance of the plant, with the appearance of spots, chlorosis, destruction of tissues, or organs or of the whole plant, decay, chancres, gills and descending death Or they may be internal, such as histological alterations, located in cells and tissues and physiological alterations that cause an increase in perspiration, immobilize nutrients and reduce the photosynthetic rate (Benítez, 2012).

In some cases, it is relatively easy to identify the causal agent of a fungal disease when the symptoms are characteristic and unique to it, reducing the possibility of error (Monroy, 2013). However, for diseases with similar symptoms, the identification can be complex; Thus, phytosanitary diagnoses provide alternatives for the timely identification of phytopathogens, avoiding production losses and economic losses (Sampietro et al., 2010).

The identification of an infectious agent through phytosanitary diagnoses is an irreplaceable tool for the elaboration of integrated disease management programs, resulting in a practical and simple action that allows to build the knowledge of the producers as a fundamental base in the search for alternatives of Solution to their phytopathological problems (Moreno et al., 2008). It is for this reason that this research consisted in analyzing samples from different cultures to determine the causal agents of the main fungus diseases that are presented in the Guanajuato shallow area, seeking to determine some strategies of timely prevention and control that the producers can implement before establishing their crops.

Methodology

Samples from plants. Samples from leaves, stems or roots of plants were disinfected with a solution of commercial sodium hypochlorite 3% and rinsed in triplicate, then placed in a humid chamber for the growth and development of pathogens. With a dissecting needle samples of already sporulated fungi were taken on the vegetal material, being placed under observation under the microscope; A portion of the plant containing typical lesions of the disease was further disinfected and allowed to dry and part of the tissue was deposited in Petri dishes containing Bioxon's Potato Dextrose Agar (PDA), Carrot-Agar (Z. AGAR) 200 g Carrot, prepared with 15 g Agar-Agar acidified with 14 ml of 10% tartaric acid, pH 5.6 ± 0.2; Agar with juice V8 (PARN) 80 ml juice V8, 15 g Agar, 3 g Calcium carbonate CaCO3, 0.27 g Ampicillin, 1.4 g tartaric acid, and 0.10 g Rivazan (PCNB), and Diacid Agar Pentachloronitrobenzene (PCNB) 20 G Agar agar, 1 g of potassium phosphate monobasic (KH2 PO4), 0.5 g of magnesium sulphate heptahydrate, 1 g of PCNB (Rizavan) and 1 g of streptomycin sulphate (Donald et al., 1996).

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The boxes were incubated for 96 hours at a temperature of 22 ± 1 ° C for optimum growth of the pathogens (Tsao, 1970).

Soil samples. 41 soil samples were obtained with established culture and 22 samples of bare soil, which were dried in the shade for 48 hours, crushing the lumps with a roller. Ten subsamples of 10 g of soil were then weighed and placed into 250 ml flasks, adding 150 ml of water, and shaking for 45 min, then 100 µL aliquots were taken and seeded in different culture media. For soils with previous plantings and to establish grasses, the following were used: Peat Agar (PPA): 15 g Peptone, 1 g Potassium phosphate monobasic, 0.5 g Magnesium sulphate heptahydrate, 1 g PCNB and 1 g Streptomycin sulphate; 20 g Dextrose, 5 g Potassium phosphate KH2 PO4, 2 g NaNO3, 0.5 g Magnesium sulphate MgSO4 7H2O, 1 g Yeast extract, 0.2 g at 1% FeSO 4 .7H 2 O , 20 g Agar, 1 g Streptomycin Sulfate and 0.0065 g of Dichloran, and for Dichloran Agar-Agar (DCPA) medium: 15 g. Agar agar, Bacto peptone, 1 g Dibasic Potassium Phosphate, 0.5 g Magnesium Sulphate Heptahydrate, 2 mg Botran (Dichloran) and 0.2 g Chloramphenicol. The PARN medium described above was also used.

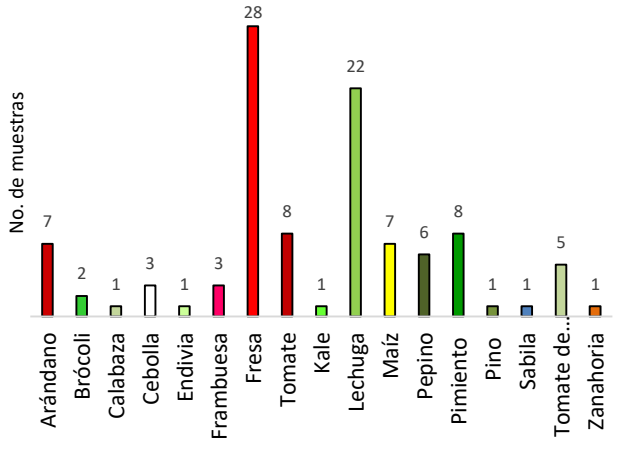
In soils for the establishment of Solanáceas, Brasicáceas or of horticultural families. PPA, DCPA and PARN media were used which were incubated for 96-120 hours at a temperature of 22 ± 1 ° C for optimum growth of the pathogens.

Water Samples. Irrigation water samples were seeded directly into boxes with PDA and Sabouraud culture medium containing 50 mg of streptomycin. The water was placed in 100 ml flasks, which were kept under agitation for 15 minutes, taking 100 µL aliquots by sprinkling in the culture media DCPA, PPA PARN and Z AGAR.

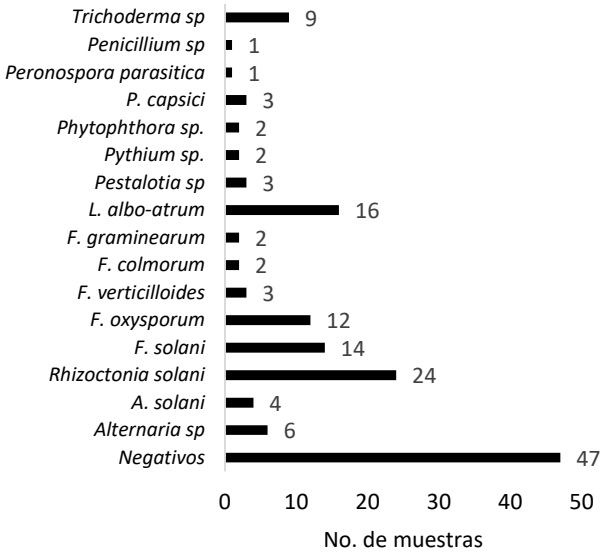
The morphological identification was made through observations under the microscope of the characteristics of shape, color, size and arrangement of spores, absence or presence of sporozoites, sclerotia or stroma in different media, mycelium and colony growth rate, as well A phytopathogenic fungi reference manual from CESAVEG (2011) was used.

Results

Sample from plants. 107 plants from 17 crops were analyzed (Graph 1). The strawberry was the crop with the highest number of samples, followed by Lettuce. R. solani was the most frequently identified pathogen in 24 samples obtained from six broccoli, onion, strawberry, raspberry, lettuce and maize cultures, Lecanicillium albo-atrum was isolated from 16 strawberry, pine and raspberry samples, F. solani Of 14 samples of broccoli, onion, strawberry, raspberry, lettuce and pepper, F. oxysporum of 12 samples of onion, raspberry, tomato, cucumber, pepper and pear tomato. However, Trichoderma sp not considered a pathogen was isolated from 9 samples from strawberry and lettuce (Graph 2).

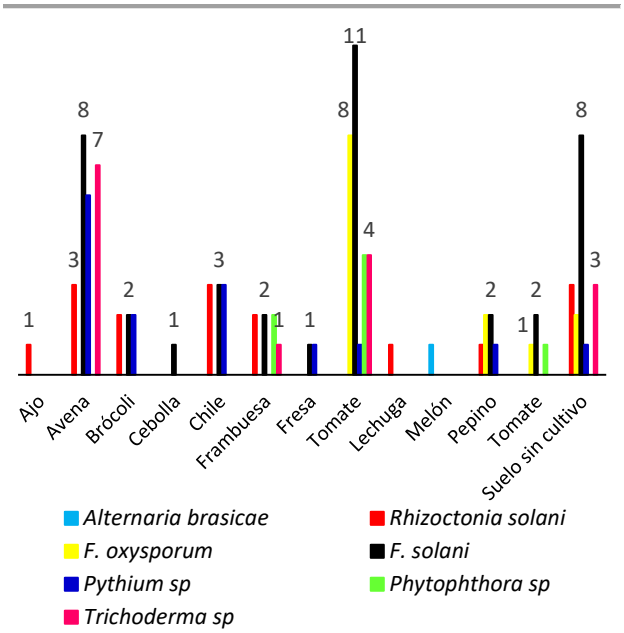


Graphic 1 Cultures analyzed for the identification of phytopathogenic fungi**.**



Graphic 2 Frequency of phytopathogenic fungi in 107 samples from plants.

Samples from soils. Fusarium oxysporum (13), Phytophthora sp (7), Trichoderma sp (15), Trichoderma sp. (15), and Rhizoctonia solani And Alternaria solani (1). The soils with the most susceptible crops were tomato and oats; however, on plant soil, phytopathological problems were also present (Graph 3).

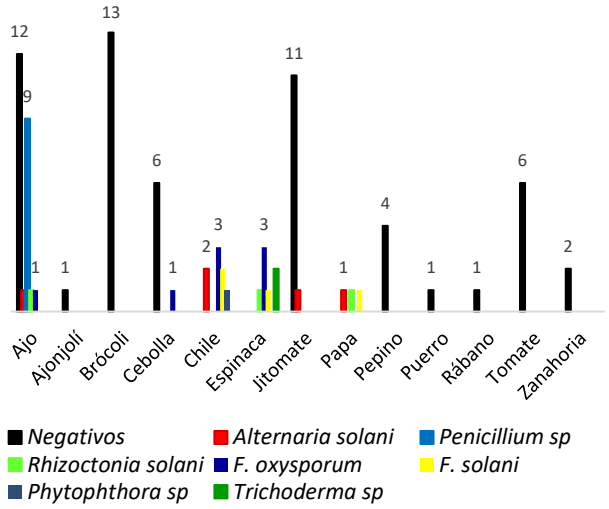


Graphic 3 Presence of fungi in soil samples.

Seed samples. A total of 75 samples from different crops such as garlic, sesame, broccoli, onion, tomato, chili, etc. were analyzed. Of which 57 were negative while, in 18 of them, frequent pathogens such as Penicillium sp (9) and Fusarium oxysporum (8) were found, and to a lesser extent the presence of Alternaria solani, Rhizoctonia solani, F. solani, Phytophthora sp and Trichoderma sp (Chart 4).

The literature reports that about 1,500 organisms have been found in seed lots of approximately 600 plant genera (Donald, et al., 1996), so it can be concluded that most of the pathogens associated with seed can be transported by itself, however, not all microorganisms found in it are causing disease. The effectiveness of the transport of pathogens and the transmission of diseases by the seed depend on a series of biotic and abiotic factors. In general, the causes of pathogen transmission increase when the inoculum is within the seed. The main genera associated with it are R. solani, F. oxysporum, F. solani, V. dahliae, Alternaria solani, Pythium sp, Macrophomina sp., Penicillium sp. And Aspergillus sp. (Agrios, 2008).

Also, the complex of diseases commonly known as Dampin off (Pythium sp., Phytophthora sp., Rhizoctonia sp., Sclerotium sp.) Can be disseminated through infected seed (Jayalakshmi, et al., 2009), so a practice Recommended is to analyze the seed before sowing for both seedling production and direct sowing, it is also recommended to immerse the seed in a solution of 108 conidia of Trichoderma harzianum, which is an effective antagonist deuteromycetes for the control of phytopathogenic fungi Of seed, plant and soil (López et al., 2010).



Graphic 4 Mushrooms found inside the seed.

In the six tray samples only the presence of Fusarium oxysporum was found in one of them and in the three samples of irrigation water Pythium was found in one of them.

It is important to mention that in order to ensure a good harvest, microbiological analyzes of soil, seed, plant and water must be carried out before establishing the crop as a preventive measure in the onset of diseases, which can occur both in developing crops As in the middle of the crop, care must be taken in the spread of pathogens within the farm, because these can remain for long periods in the soil through resistance structures such as chlamydiospores and sclerotia (Martínez-Scott, 2008) . It has sometimes been observed that when soils are infested by Fusarium oxysporum, the structures are carried by the irrigation water or leached to the aquifers and the water is contaminated, causing the plants watered with the same to wilt and stop growing (Ramírez et al., 2009). This research was of great help to the producers of the region of the guanajuatense bajío, because from the results it was possible to take preventive measures and to execute corrective actions to avoid economic losses.

Conclusions

Of the 245 samples analyzed, a total of 275 pathogens were obtained in 130 samples grouped into 10 genera and 14 species. Strawberry cultivation showed the highest number of pathogens such as Rhizoctonia solani, Verticillium albo-atrum and fusarium oxysporum, and Alternaria solani and Phytophthora sp. According to the results obtained, the fungus Rhizoctonia solani was the pathogen that was most frequently found in both plants and seed and soil samples. In germination trays samples were mainly identified to Fusarium oxysporum and in irrigation water to Pythium sp and Trichoderma sp., the rest gave negative to fungi.

Aknowledgement

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