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MORPHOLOGICAL IDENTIFICATION, PATHOGENICITY AND *IN VITRO* CHEMICAL CONTROL OF THE CAUSAL AGENT OF CROWN ROT IN STRAWBERRY

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Abstract: Strawberry crown rot *Fragaria x ananassa* duch it causes necrosis of the tissue in the axis of the leaves and rot of the crown, which ultimately ends with the collapse of the plant. In the present investigation, the morphological identification of the causative agent of diseased plants from Zamora, Michoacán was carried out, the pathogenicity was also evaluated and *in vitro* control tests were carried out in which five fungicides were used (Azoxystrobin 25SC 0.4 mL PF/L of water, thiabendazole 60WP 0.6 g PF/L, pyraclostrobin 25CE 1.0 mL PF/L, cyprodinil+fludioxonil 62.5WG 1.0 g PF/L, and azositrobin+propiconazole 20SE 0.4 mL PF/L), a completely randomized experimental design was used with five treatments, five repetitions and an absolute control, using two methods; poisoned crop and filter paper. *Neopestalotiopsis* was identified sp. as a causal agent of crown rot in strawberry. The isolates were highly virulent showing symptoms, after four days, in 100% of the inoculated plants. In the *in vitro* control tests with both methods, the treatment with the highest percentage of mycelium inhibition was Cyprodinil+fludioxonil.
Keywords: *Fragaria x ananassa,* crown rot, *Neopestalotiopsis* sp. Cyprodinil+fludioxonil.

INTRODUCTION

Mexico is the third largest producer of strawberries worldwide. In 2020, 861,300 tons of this fruit were produced, of which 207,100 tons were exported to 18 countries on three continents. It must be noted that Mexico exceeded its strawberry exports to Belgium in 2012, to the Netherlands in 2016, to the United States in 2019 and to Spain in 2020 (SAGARPA 2020). The strawberry is a fruit for export and of high nutritional value, but it is highly susceptible to the attack of pathogens, requiring an intensive use of pesticides in its production; Furthermore, this chemical control is highly expensive and harmful to users and the environment (Godoy, 2018).

In Mexico, they have reported that limiting phytosanitary factors for strawberry cultivation are fungi and pseudofungi that cause root diseases, highlighting strawberry crown rot, which consists of wilting and gradual death of the plant. *neopestalotiopsis* sp. It causes necrosis of the tissue in the axis of the leaves, parts of the upper crown and rot of the basal crown in strawberries, which in turn causes the collapse of the plant (Van Hemelrijck *et al.*, 2017).

The disease caused by *Neopestalotiopsis* sp. It is a new problem, before it was only considered as an opportunistic and saprophytic fungus; however, for a few years it has been found strongly attacking berry crops, such as blueberries, causing regressive deaths and cancers at the base of the stems. This fungus has also been found attacking raspberries and blackberries, but the strongest problem is being detected in strawberry crops. (Rebollar, 2020).

"The symptoms in this crop used to be confused with other fungi, such as *Fusarium* spp., *Pythium* sp. or *Phytophthora* spp; however, when inquiring, the *Neopestalotiopsis fungus always appeared* sp., Likewise, this phytosanitary challenge is not only reported in the strawberry production regions of Mexico, but in various parts of the world, such as the United States, Argentina, France, among other countries. (Rebollar, 2020). In Mexico, it is highly relevant to take preventive measures against this new pathogen and carry out a thorough identification of the fungus, since its genus is known, but not its species. (Rebollar, 2020).

Strawberry crown rot is the main disease of this crop in the state of Guanajuato, Mexico, and causes losses of up to 100% of production (Ceja, 2008).

Given the problems and losses caused by

crown rot in strawberry and whose control has not been successfully achieved, causing the indiscriminate use of fungicides; The objective of this study is to identify the causal agent of crown rot in strawberry, in addition to evaluating its pathogenicity and finding the most effective chemical product for its control under in vitro conditions.

MATERIALS AND METHODS

MATERIAL COLLECTION

For the collection of diseased plant tissue, directed samplings were carried out in different farms cultivated with strawberries in the municipality of Zamora, Michoacán. At each sampling point, 8 plants with symptoms of crown rot were collected, the samples were placed in paper bags to prevent increased humidity and the development of saprophytic fungi. The study was carried out in the Laboratory of the Faculty of Agrobiology, Presidente Juárez.

ISOLATION

A disinfestation process was carried out, which consisted of superficially cleaning the infected material (leaves and crown) with tap water to eliminate possible contaminants present, cuts of approximately 2 x 2 millimeters wide and long (from the area of the progress of the symptom), they were submerged in 2% sodium hypochlorite for 120 s followed by rinsing with sterile distilled water, performing this procedure 3 times, they were placed on sterile paper towels to absorb excess moisture and in the flow hood laminar, five tissue sections distributed equidistantly were placed in Petri dishes with PDA culture medium.

They were duly labeled and sealed with clean pack paper, they were placed in an incubator at a constant temperature of 25 °C with a relative humidity of 100 %, they were monitored every 24 hours to perform hyphal tip purifications as soon as growth of the hyphae was observed. mycelium.

MORPHOLOGICAL IDENTIFICATION

Once the pure colonies were obtained, the morphological identification was carried out with the help of the specialized taxonomic keys of Barnett and Hunter (1998), aspects such as the shape of the colony, elevation, pigmentation of the medium and growth were evaluated, microscopically the type was evaluated. of mycelium (coenocytic or septate) and the presence of reproductive structures such as acervuli and chlamydospores of the asexual phase (anamorph) and the presence of ascocarps and ascospores of the sexual phase (teleomorph).

PATHOGENICITY TESTS

To evaluate the pathogenicity of the strains obtained, they were inoculated directly and indirectly; The first consisted of making small wounds on the crown of the strawberry seedling with a 0 (zero) entomological pin, to later place PDA discs with mycelium of approximately one centimeter in diameter on the wound, covered with cotton moistened with water. sterile distilled. The second method used was spraying, in which a suspension of conidia was made at a concentration of 1 x $10^{6,}$ which was adjusted using the Neubauer chamber with the following equation $Cell\ concentration = \frac{Number\ of\ counted\ cells}{Number\ of\ tables} \times 10{,}000)$, once adjusted to the desired concentration, it was Small wounds were made on the stems and crown of the strawberry plants and the suspension was sprayed with a manual sprinkler; finally, the plants were covered with a plastic bag as a humidity chamber for 3 days.

IN VITRO CONTROL

For this test, five chemical fungicides were used (Azoxystrobin 25SC 0.4 mL

PF/L of water, Thiabendazol 60WP 0.6 g PF/L, Pyraclostrobin 25CE 1.0 mL PF/L, Cyprodinil+fludioxonil 62.5WG 1.0 g PF/L, and Azositrobin+propiconazole 20SE 0.4 mL PF/L). With the doses of the formulated product, each one with different modes of action and an absolute control, without fungicide.

For the tests, the fungicides were added in two ways, the first was to add them to the PDA (Potato dextrose agar) culture medium when it was at a temperature of 45-50°C. This mixture was homogenized by gently shaking it and 16 mL was poured per Petri dish. After one hour of solidification and cooling, an agar disk with mycelium (5 mm in diameter) of isolation of *Neopestalotiopsis was placed in the center of the Petri dish.* sp. to be tested, for the second method they were placed in Petri dishes with culture medium and 5mm diameter filter paper discs impregnated with each of the fungicides previously diluted at the recommended doses were placed equidistantly.

The Petri dishes of the control treatment contained only PDA culture medium. The tests were kept in an incubator at 24°C. A completely randomized experimental design was used, five treatments, five repetitions and an absolute control. The measurements of each treatment were made every 24 hours. until the witness covered the box. Statistical analysis, Tukey tests ($P<0.05$) were performed, the data was analyzed in the JMP program.

RESULTS AND DISCUSSION

MORPHOLOGICAL IDENTIFICATION

The colony grew in a circular shape, presenting a cottony white color, forming several rings. The medium did not change color until one week after the colony had reached the edge of the plate, then a yellowish color was observed. The mycelium produced points of shiny black color, hard consistency and irregular shape that, according to Montiel and Avelar (2001), correspond to cirrus, which are masses of conidia united by a gel. I present cylindrical conidia of between 22 to 30 µm with a slight curvature at the ends, with one to four septa with characteristic color of the intermediate septa from dark brown to semi-transparent olive while the color of the external cells is transparent, they presented one to four basal appendages and 1 apical appendage, these characteristics agree with those described for *Neopestalotiopsis* sp. as reported by Obregón *et al.* (2018), Jeewon *et al.* (2002) and Garrido, (2007) (Figure 1).

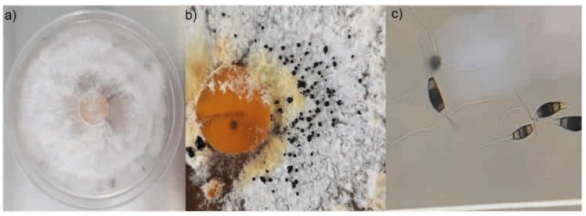

Figure 1. a) *Neopestalotiopsis* colony sp., cottony white, forming several rings, b) Cirrus present in *Neopestalotiopsis colonies* sp., shiny black in color and irregular in shape, c) conidia with 4 dark brown to olive septa in the center and two to three basal appendages and one apical.

PATHOGENICITY TESTS

Greater severity was observed when inoculating by spray method since the symptoms became visible four days after being inoculated, affecting up to 80% of the plants in two weeks, contrary to inoculation by means of mycelium fragments, that the symptoms could be observed a week later, the plants presented the characteristic symptoms of *Neopestalotiopsis* sp., described by Garrido (2007), Núñez *et al.,* (2017); Van Hemelrijck *et al.*, (2007) and Ceustermans *et al.,* (2015) where they point out that the disease produced by this pathogen in strawberry affects the basal zone of the plants, necrosis of the crown

was observed, turning the leaves reddish and a moderate collapse of the inoculated plants (Figure 2)..

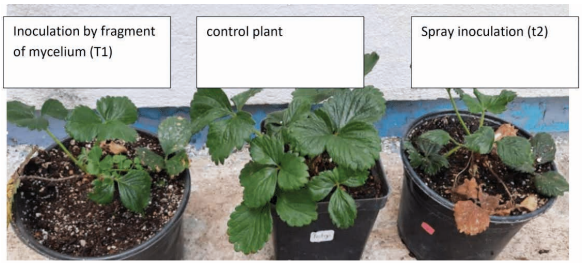

Figure 2. Comparison of symptoms in strawberry plants inoculated directly and indirectly, plus a control.

To finish with Koch's postulates, one of the inoculated seedlings was taken and sowed again in PDA to verify that it was *Neopestalotiopsis.* sp.

CHEMICAL CONTROL

Significant differences were observed between treatments with both methods. The fungicide that showed the highest percentage of mycelium growth inhibition was Cyprodynil + Fludioxonil, the results coincide with what was reported by (Obregón *et al.,* 2018), where they mention that this active ingredient has greater inhibition efficiency against the fungus *Neopestalotiopsi*s sp (Figure 3).

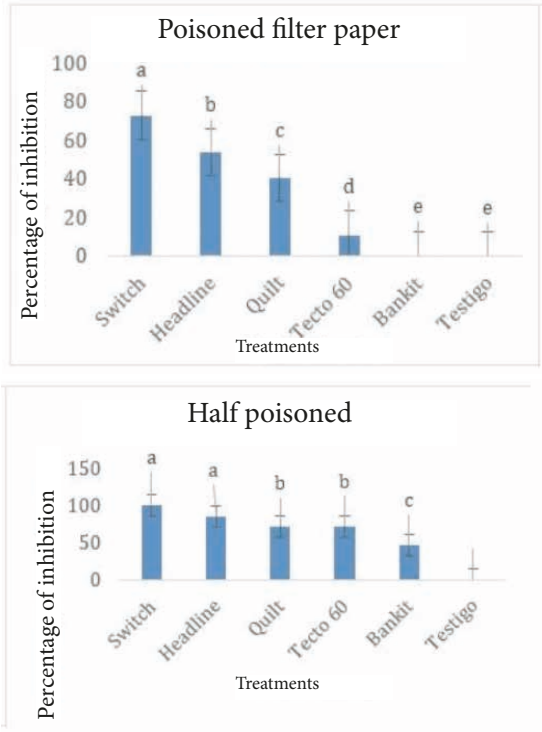

Figure 3. Percentage of inhibition of mycelium with the filter paper method and poisoned medium. Different letters indicate statistically significant differences according to Tukey's test (P< 0.05).

CONCLUSIONS

Neopestalotiopsis sp. It is the causal agent of crown rot, isolated from strawberry. *neopestalotiopsis* sp. It turned out to be very virulent when attacking this crop.

Cyprodinil+fludioxinil was the best product for the *in vitro control* of *Neopestalotiopsis* sp.

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