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Pathogenic capacity of *Pestalotia longisetula* Guba reported for the first time on strawberry (*Fragaria ananassa* Duch.) in Morocco

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ABSTRACT

In the spring season of 2011, fungal isolation under laboratory conditions from senescent strawberry plants of Festival variety collected from a farm in Dlalha (Moulay Bousselham, Northwestern Morocco) revealed the presence of two isolates of Pestalotia longisetula. The Koch's postulate was verified by inoculating healthy leaves of Festival and Sabrina strawberry varieties using mycelia discs and conidia suspension. Severity index on Festival and Sabrina leaves reached respectively 85 - 77.5% and 73.2 - 69.8% for inoculation by the first technique. The inoculation by the second technique revealed that the severity index of the two isolates of P. longisetula (PD and PF) were superior on leaves of Festival, respectively 68 - 63.1% against 56.8 - 50.5% on Sabrina leaves. The lowest conidia production was observed on Sabrina leaves, inoculated with PF isolate conidia suspension (1.58 10^5 conidia. cm^{-2}). Elevated conidia production was recorded on Festival leaves, 2.81 10^5 conidia. cm^{-2}, inoculated with the PD mycelial disc. This pathogen was also able to infect ripe strawberries of Festival and Splander varieties. The PF isolate showed the greatest capacity to induce superficial and depth lesions respectively 16.1 and 16.6 mm and to produce conidia, 9.4 10^5conidia. cm^{-2}, on Splander variety which seems to be most susceptible to infection. This was the first report of P. longisetula showing the capacity to infect strawberry leaves and fruits in Morocco.

Key words: *Pestalotia longisetula, Fragaria ananassa, leaves, strawberries, pathogenecity, Morocco.*

INTRODUCTION

Strawberry (*Fragaria ananassa* Duch.) is one of the most important vegetable crops for local consumption and exportation in Morocco. However, the possible causes of introducing strawberry diseases were the increasing cultivation these last decades and the importation of the planting material due to deficiency of certified planting material. Various kinds of diseases were reported to occur both on strawberry plants in the field, during shipment and storage time as Verticillium wilt[22,28] anthracnose crown rot and root necrosis[10], leaf spots[7] and fruit rots as gray mold induced by *Botrytis cinerea* [3], strawberry leak caused by *Rhizopus* spp.[31]. Unfortunately, previous isolation from rotted fruits[9] revealed severe infections related to fungi as *Alternaria* sp., *Botrytis cinerea*, *Rhizopus stolonifer*, (*Pestalotiopsis* sp.) and *Pestalotia longisetula* occurring at higher frequency than others. A frequency of *Pestalotiopsis guepinii* in the examined petioles was 13% from aboveground parts of symptomless plants[24]. In USA, *Pestalotia longisetula* caused a cortical decay of petioles and stolons of strawberry[25] and was isolated from diseased strawberry root in China[37]. In 2010, a sickly strawberry plant parts collected from two strawberry farms in Moulay Bousselham (Northwestern Morocco) yielded numerous fungal species with varied frequency without any member of *Pestalotiopsis* species[23]. Though, in the spring season of 2011, fungal isolation from different parts of a sample strawberry plants showing chlorosis symptoms (Figure 1) collected during the prospecting assays performed in the same locality revealed the presence of *P. longisetula* recorded for the first time in Morocco accompanied by common fungal invaders as *Botrytis cinerea*, *Alternaria alternata*, *Cladosporium* sp. and *Fusarium* sp.

Thus, this study was conducted to verify the Koch's postulate of *Pestalotia longisetula* on the strawberry leaves as well as on the strawberry fruits.

MATERIALS AND METHODS

Pathogen Isolation

Two isolates of *Pestalotia longisetula* (PF and PD) were isolated using the technique of a modified humid chamber[2] respectively from leaves and stem of chlorotic strawberry plants of Festival variety brought from a farm in Dlalha (Moulay Bousselham, Northwestern Morocco) were cultivated on PSA medium (Potato Sucrose Agar: 200 g potato, sucrose: 20 g, Agar-agar: 15 g, distilled water: 1000 ml) and incubated in the dark for five days at 28°C.

Pathogenicity test on the strawberry leaves

The healthy leaves of two strawberry varieties, Festival and Sabrina were inoculated using two techniques. The surface of sixty leaves was disinfected with 5% sodium hypochlorite, washed with sterile distilled water and dried on a filter paper. Fifteen leaves were inoculated with 5 mm mycelial discs of the fungus placed in the middle of the intact leaves, the other fifteen ones were inoculated with the conidial suspension adjusted to a final concentration of 10^5 conidia. mL^{-1} with sterile distilled water containing 0.05% Tween 20 and 5% gelatin. Thirty leaves were used as a control, the half of them were inoculated with distilled water containing Tween 20 and gelatine and the other half with only PSA medium discs. Every bunch of three leaves was placed in 120-mm Petri dish containing slide and sterile distilled water. Inoculated leaves were incubated at room temperature (24°C) in the darkness. The diseased leaf area was scored after 15 days of inoculation using the scale of Stover modified by Gauhl et *al*.,[11] : **0**= No symptoms ; **1** = -0.5% of the limbus with symptoms ; **2** = 0.6 to 5% of the limbus with symptoms ; **3** = 6 to 15% of the limbus with symptoms ; **4** = 16 to 30% of the limbus with symptoms; **5** = 31 to 50% of the limbus with symptoms ; **6** = 51 to 80% of the limbus with symptoms ; **7** : 81 to 100% of the limbus with symptoms.

The severity index (IS) of disease was calculated using the formula:

$$IS=(\Sigma nb/(N-1) \times T) \times 100$$

n= Number of leaves for each degree of the scale.

b= Degree of the scale.

N= Number of the degrees used in the scale.

T= Total number of the scored leaves.

The conidia production (Conidia. cm^{-2}) of *Pestalotia longisetula* on the inoculated strawberry leaves was estimated according to the technique of Hill and Nelson[13]. Ten days after inoculation, the leaves those had shown lesions were cut into pieces of 1 cm^2 and placed in 90 mm Petri dishes on three filter paper discs moistened with sterile distilled water. The dishes were incubated for 48 hours under continuous fluorescent lighting. Then each fragment was placed in a test tube containing 1 mL of sterile distilled water and agitated by a vortex mixer for 2 min. The conidia of the pathogen were counted using a Malassez slide under an optical microscope at magnification × 100 with 10 counting of each sample.

Pathogenicity test on the strawberry fruits

Ripe strawberries of Festival and Splander varieties were washed in running water and surface disinfected by immersion for 1 min in 95% ethyl alcohol followed by 15 min in a 0.5% sodium hypochlorite solution. They were rinsed three times in sterilized distilled water and placed aseptically in disinfested plastic containers (5 cm × 7 cm), one fruit per container. Lids were placed loosely on the container to avoid the moisture excess but maintain sterile conditions. The berries were inoculated artificially at a 4 mm cutting injury with a 5 mm mycelial disc of *Pestalotia longisetula* culture. Controls received only 5 mm mycelial disc of PSA culture. The containers containing berries were then placed in room temperature in the laboratory at 24 ± 1°C. There were 10 ripe fruits in each variety.

Measured parameters

After 7 days of incubation, the perpendicular diameters and the depth of the rot lesions were measured by a double decimeter.

After 15 days, inoculated strawberries were removed from their plastic containers and pieces of 1 cm^2 were cut in the flesh fruit level to estimate the conidia production by the technique of Hill and Nelson[13] as it was used before on the leaves.

RESULTS AND DISCUSSION

P. longisetula isolated from strawberry plants collected in strawberry farm in Moulay Bousselham (Northwest Morocco) during the spring 2011 was identified basing on the morphological characters described by Steyaert[29] and Guba[12].

After incubation, numerous acervuli were especially produced in chlorosis leaves of strawberry plants (Figure 2A). On PSA, aerial mycelium of *P. longisetula* was white, more branched and golden brown in reverse attaining 70 mm of a diameter after 7 days in dark at 24°C (Figure 2B) and acervuli were produced in old culture. Conidia (20 – 28 × 6-8.3 µm) were fusiform, straight or slightly curved and five celled. The darker median cells were three celled with a thick wall. Normally, the upper two cells were brown with a darker band at the septa between them, while the lowest cell was lighter colored. The apical and basal cells were conical in shape, thin walled and colorless. Appendages appeared at the apex and base. There were two to four appendages (19.5 – 31.5 to 37 µm length), mostly three. Basal appendage (6.5 - 9.5 µm) was single and centric (Figure 2C).

After 15 days of inoculation by a mycelial disc, a dark brown zone was developed in the surface of the inoculated leaves (Figure 3A). In the inoculated leaves by the conidial suspensions, the lesions enlarged to eventually cover several centimeters and after 20 days the fungus began to form conidiomata as minute black dots, the acervuli scattered on the leaf spot (Figure 3B). The healthy strawberry leaves have not shown any symptoms (Figure 3C).

Disease severity appeared at different degrees according to the isolates and the studied variety. 15 days after inoculation, the estimated disease severity index on Festival and Sabrina strawberry leaves were respectively 85 - 73.2% and 77.5 - 69.8% after inoculation by mycelial discs of PD and PF isolates (Figure 4A). After inoculation with conidial suspension, the severity index was superior on leaves of Festival compared to those of Sabrina respectively inoculated with PD and PF 68 - 63.1% and 56.8 - 50.5% (Figure 4B).

The isolate PD of *P. longidetula* produced conidia on the leaves of Festival and Sabrina strawberry varieties inoculated by mycelial disc respectively 2.81 and 2.24 10^5 conidia. cm^{-2} (Figure 5A). A less conidia's number was produced on leaves of Festival and Sabrina strawberry varieties inoculated by the PD isolate, after the inoculation with the conidia suspension, respectively 2.14 - 1,74 10^5 conidia. cm^{-2} (Figure 5B). The conidia production of PF showed no significant difference between Festival and Sabrina leaves inoculated by conidia suspension (Figure 5B). On contrary, it produces 2.57 10^5conidia. cm^{-2} on Festival compared to Sabrina leaves (1,9 10^5conidia. cm^{-2}) inoculated by mycelia disc (Figure 5A).

Similarly, Sharifi et *al.*[26] proved the pathogenicity of *P. longisetula* against strawberry plant where stems and leaves developed a symptom of infection five days after inoculation. In Brazil, this fungus was capable to induce leaf spots on strawberry plant[4].

Wounded fruits inoculated with mycelial discs of PSA have not shown any symptoms. Rots started with watery surface lesion around the inoculated wound and on the depth of berries (Figure 6, c_1-c_2). The infected tissue are initially, discolored, showing pale brownish color and softening, then, are covered with dense aerial mycelium within one week from inoculation by mycelial disc of PD (Figure 6, a_1-a_2) or PF (Figure 6, b_1-b_2). 15 days after inoculation, the aerial mycelium covers the entire berry and droplets of liquid containing spores are scattered over it (Figure 6, d_1). However, numerous black acervuli erupt through the epidermis around the injured area in the presence of little mycelium of the pathogen in front of *Botrytis cinerea* (Figure 6, d_2). Diameter of lesions on the surface (Figure 7A) and depth (Figure 7B) of injured Festival fruits inoculated with the mycelial disc of PD were respectively 12 mm and 13.8 mm

compared to those of PF 13.2 mm and 14.3 mm. On Splander variety, PD produced superficial lesion diameters equal to 10.2 mm on the surface and 12.1 mm on the depth. On the other side, PF was more virulent; the lesion diameters reached 16.1 mm on the surface and 16.6 mm on the depth.

15 days after inoculation, conidia production of PD was respectively 6.5 and 4.9 10^5 conidia. cm^{-2} on the injured Splander and Festival berries (Figure 7C). While PF produced 9. 43 10^5 conidia. cm^{-2} on Splander and 8. 16 10^5 conidia . cm^{-2} on Festival (Figure 7C).

Pestalotia rot of strawberry fruits caused by *P. longisetula* Guba, was first recorded in Israel[15], USA Howard and Albregts, (1973) and then in India[27]. In Egypt, it is a virulent fungus under field condition causing fruit rot and severe damage especially under low temperature and higher relative humidity as well as rain and cool season[9]. This virulence has been also indicated towards four different hosts under laboratory conditions, it was to infect apricot, peach, guava and tomato fruits causing fruit rots[9].

Indeed, *Pestalotiopsis* is a species rich genus occurring as pathogens, endophytes and saprophytes[16]. However, most species of *Pestalotia* are plant pathogens[35,36]. They cause leaf blights[12] in many plant species[6,20,33]. Symptoms of petiole necrosis and foliar yellowing were caused by *Pestalotiopsis clavispora* on *Argania spinosa* L. Skeels[1], leaf blight on Japanese spicebush (*Lindera obtusiloba*) caused by *Pestalotiopsis microspora*[17], leaf spot disease of *Proteaceae* caused by *Pestalotia* sp. in Zimbabwe[30]. Species may also cause fruit rots and other post-harvest diseases[19,32,33]. Several postharvest diseases are caused by *Pestalotiopsis* species, e.g., postharvest decay of mangos by *P. glandicola* (Castagne) Steyaert[32], fruit rot of grapevine by *P. menezesiana* (Bres. & Torrend) Bissett as well as *P. uvicola* (Speg.) Bissett[33], fruit rot disease on the Cheongsoo grape cultivar induced by *Pestalotiopsis* sp.[8] and soft decay of *Syzygium samarangense* fruit flesh by *Pestalotiopsis samarangensis*[21].

In Morocco, several pathogenic species of the genus *Pestalotia* were also reported as *Pestalotiopsis cruenta* witch provokes brown lesions with clear black circle on the leaves of *Chamaerops humilis*[18], *Pestalotia subcuticularis* causing lesions on the leaves of *Pyrus mamorensis*[34] and *Pestalotia fici* the causal agent of the olive leaves chlorosis and olives rot[5]. But this is the first report of the presence of *P. longisetula* in planting area of production of strawberry. Therefore, the fungus had a great ability to infect leaves and fruits in bioassays, however, it still too early to assess the potential economic importance of the disease.

Fig.1: Strawberry plants of Festival variety showing chlorosis symptoms brought from a farm in Dlalha (Moulay Bousselham, Northwestern Morocco)

Fig.2: Acervuli on the leaf fragment (A) ; colony of *P. longisetula* isolated from strawberry leaf and cultivated on PSA (B) and conidia (C)

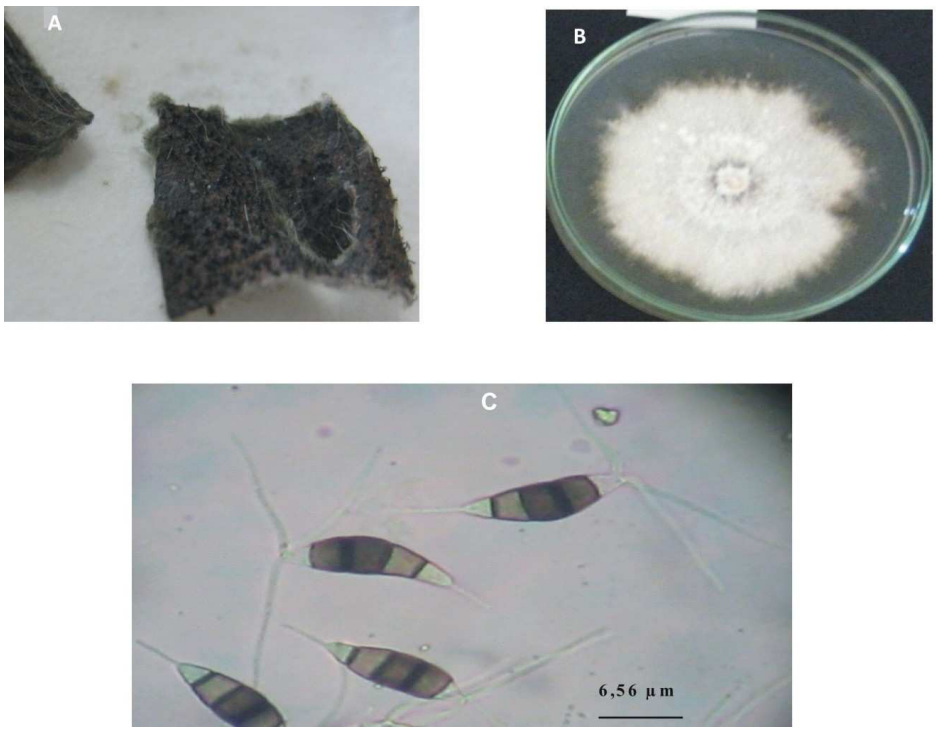
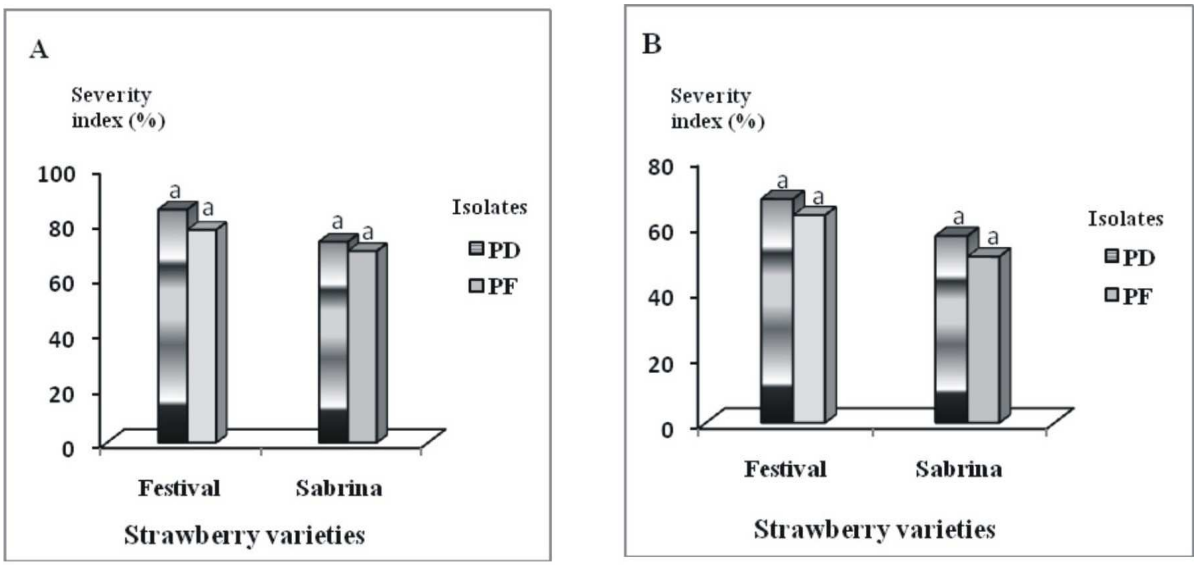


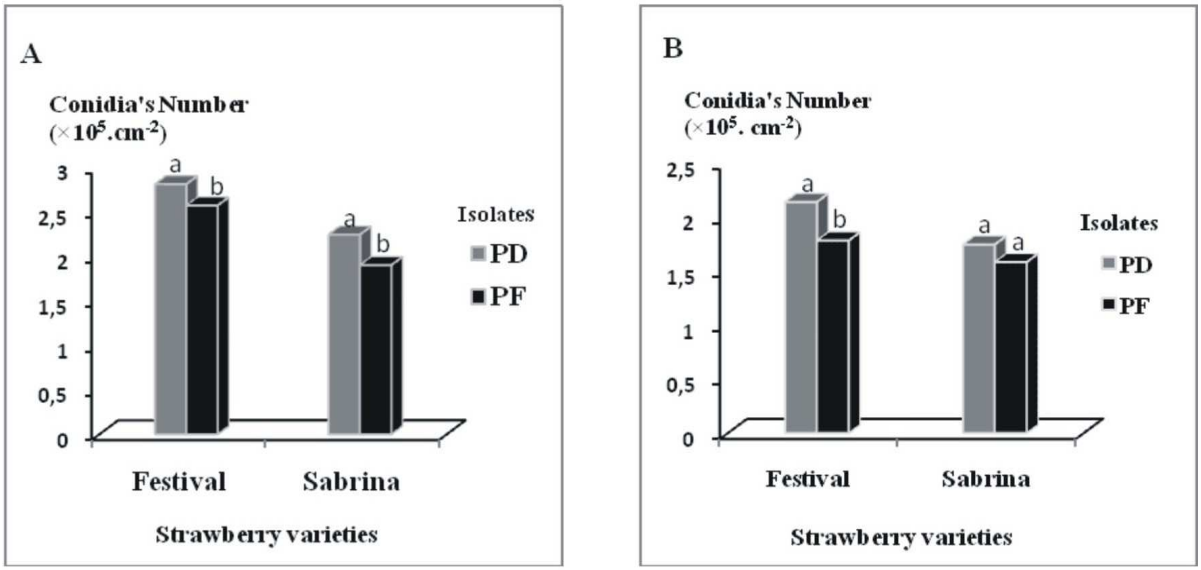
Fig.3: Lesions developed on strawberry leaves after artificial inoculation by *Pestalotia longisetula*. Inoculated leaves with mycelia disc (A) ; inoculated leaves with spore suspension (B) ; control leaves (C)

Fig.4: Severity disease on leaves of Festival and Sabrina strawberry varieties after inoculation with mycelial disc (A) and Conidial suspension (B) of *Pestalotia longisetula*



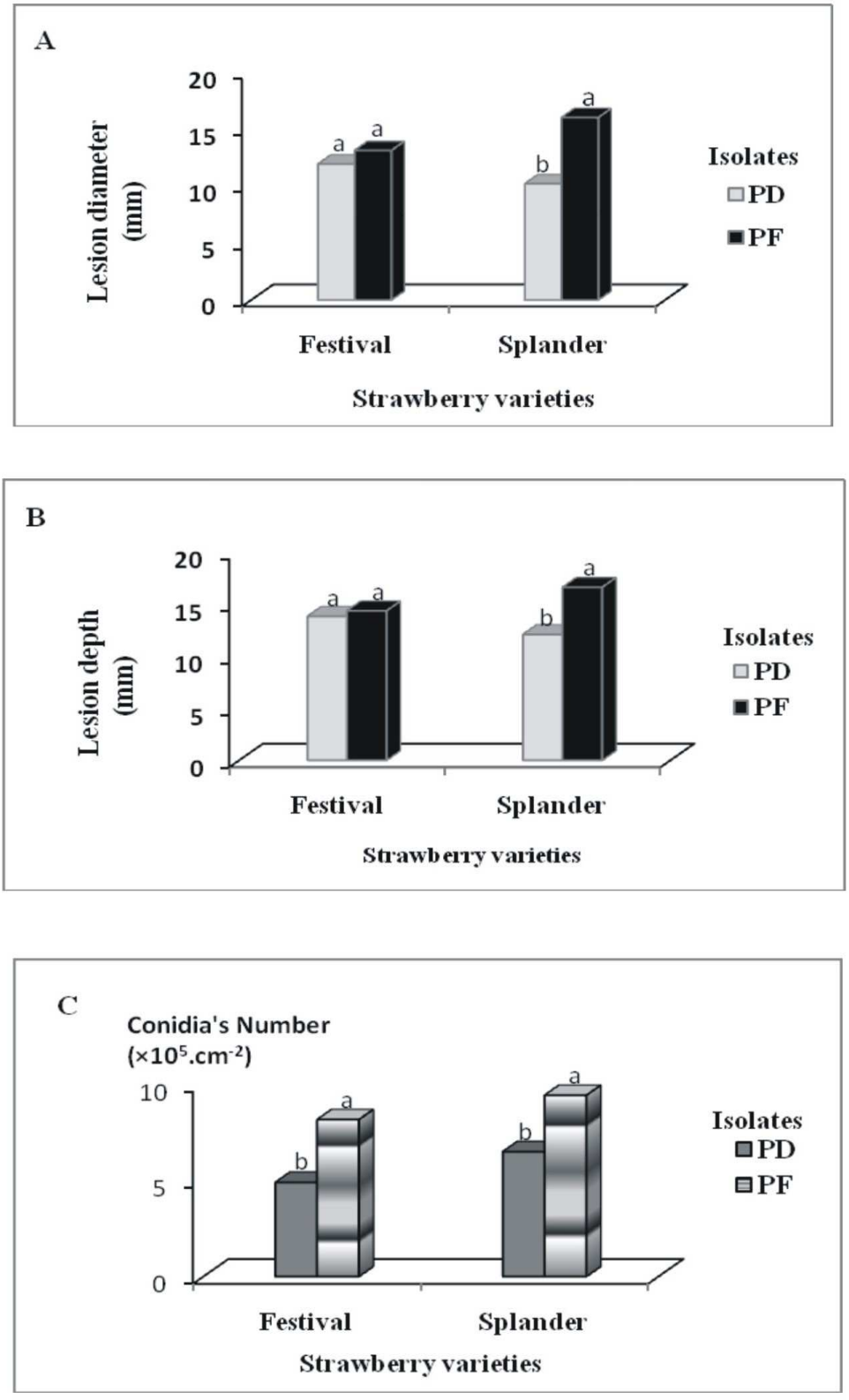
Values followed by the same letter do not differ significantly at 5%.

Fig.5: Conidia production of *Pestalotia longisetula* on the leaf surface of Festival and Sabrina strawberry varieties after inoculation with mycelial disc (A) and conidia suspension (B)



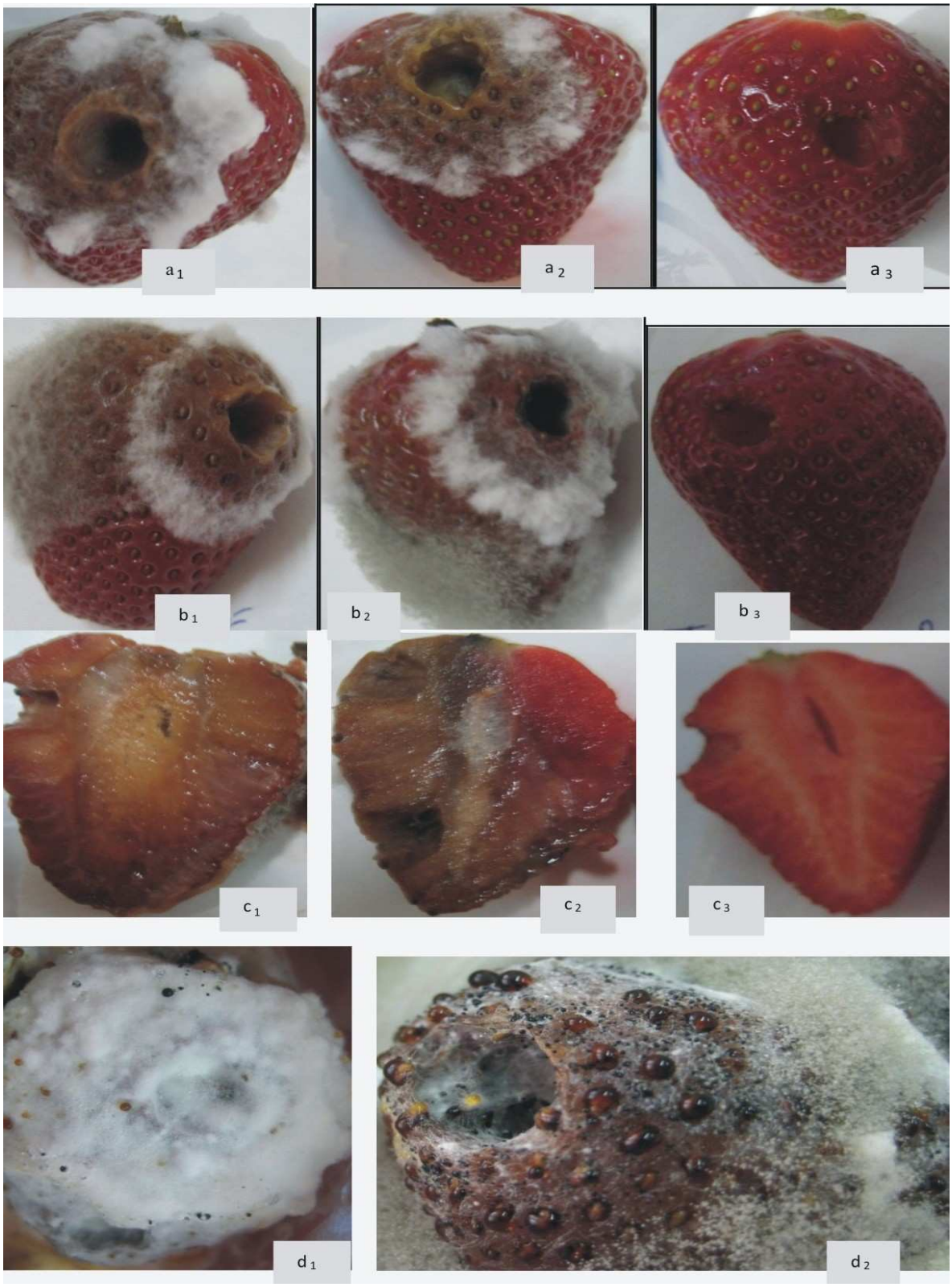
Values followed by the same letter do not differ significantly at 5%.

Fig.6: Superficial lesion diameter (A), lesion depth (B) and conidia's number (C) produced on the surface of Festival and Splander strawberries varieties



Values followed by the same letter do not differ significantly at 5%.

Fig.7: Reaction of the two strawberries varieties to the artificial inoculation with mycelia discs of *P. longisetula* isolates



Mycelial disc of PD on a injured Festival (a_1), Splander (a_2) strawberry fruit; Mycelial disc of PF on an injured Festival (b_1), Splander (b_2) strawberry; PSA media disc on an injured Festival (a_3) and Splander (b_3) strawberry fruit; the depth of the internal rot on Festival (c_1), Splander (c_2) fruit inoculated with mycelia disc of PF; inoculated by mycelia disc of PSA (c_3); aerial mycelia on fruit with acervuli of *P.longisetula* (d_1, d_2) after 15 days of inoculation.

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