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Isolation and Physiological Studies of Fungi Associated with Post-Harvest Diseases of Selected Solanaceous Fruits in Ilorin Markets, Nigeria

Isolation et études physiologiques des champignons associés aux maladies post-récoltes des fruits de certaines solanacées dans les marchés d'Ilorin, au Nigéria

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Abtract

This study was carried out to determine the fungi associated with diseases of some solanaceous crops stored in selected markets in Ilorin. The diseased sample fruits of Tomato (*Solanum lycopersicum*), Pepper (*Capsicum annuum*) and Eggplant (*Solanum melongena*) were randomly collected and isolation of fungi from the samples was carried out using Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA). Pathogenicity test and physiological studies of the isolates were performed. Total of seven fungi, *Aspergillus niger, A. terreus, A. oryzae, Colletotrichum coccodes, C. capsici, Schizosaccharomyces pombe, Penicillum funiculosum* and *Rhizopus Stolonifer* were isolated in all the sampled fruits and *C. coccodes* was present in all fruit samples. The results from pathogenicity test showed that *C. coccodes* was the most pathogenic fungus. The mycelia dry weights of *C. coccodes* in various media were 1.04g in glucose, 1.16g in sucrose and 1.13g in fructose. It was revealed that isolated fungi showed differential ability in utilizing different carbon sources.
Key words: Carbon sources, disease, isolation, mycelia, pathogenicity

Résumé

Cette étude a été réalisée pour déterminer les champignons associés à des maladies de certaines solanacées cultivées et stockées dans des marchés à Ilorin. Les échantillons de tomate (*Solanum lycopersicum*) malades, de poivre (*Capsicum annuum*) et d'aubergines (*Solanum melongena*) ont été recueillis au hasard et l'isolement des champignons a été réalisé à l'aide d'Agar de Dextrose Sabouraud (SDA) et d'Agar De Dextrose De Pomme De Terre (PDA). Le test de pathogénicité et les études physiologiques des isolats ont été effectués. Sept champignons, *Aspergillus niger*, *A. terreus*, *A. oryzae*, *Colletotrichum coccodes*, *C. capsici*, *Schizosaccharomyces pombe*, *Penicillum funiculosum* et *Rhizopus Stolonifer* ont été isolés dans tous les fruits échantillonnés et *C. coccodes* était présent dans tous les échantillons d'espèces. *C. coccodes* a été le champignon le plus pathogène. Les poids mycéliaux secs de *C. coccodes* dans divers milieux ont été de 1,04 g dans le glucose, 1,16 g dans le saccharose et 1,13 g dans le fructose. Il a été révélé que les champignons isolés présentaient une capacité différente dans l'utilisation de différentes sources de carbone.
Mots clés : sources de carbone, maladie, isolement, mycélium, pathogénicité

1. Introduction

Tomato (*Solanum lycopersicum* L.), Pepper (*Capsicum annuum* L.) and Eggplant (*Solanum melongena* L.) are nutritious fruits but highly perishable due to fungal spoilage and wastage as a result of non-availability of or poor storage conditions in developing countries such as Nigeria.

Postharvest loss includes the collective loss along the agricultural value chain, from harvest and handling (in the field) to storage and processing (Michailides et al.

2010). This loss involves not only the quantity but also the nutritional quality of the fruits. Postharvest diseases pose threat to a wide variety of crop especially in developing countries with poor storage facilities (Babalola et al. 2010; Jeffries and Jeger 1990).

The physiological activities of climacteric fruits such as tomato coupled with their high percentage moisture contribute to their short shelf life (Idah et al. 2007). Post-harvest losses of fruits are also caused by pathogens especially fungi such as *Colletotrichum coccodes*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium oxysporum* and a host of others (Al-Najada and Al-Suabeyl 2014, Chohan et al. 2016). These fungi contaminate the produce and affect their post-harvest values.

From Tomato, Matthew (2011) reported that *Aspergillus niger, Rhizopus nigricans, R. stolonifer and Mucor* spp. are responsible for its decay and out of the 22 fungal species recorded from different parts of the world on Eggplant, *Alternaria*, *Fusarium*, *Colletotrichum*, *Phytophthora* and *Phomopsis* have been reported to cause rots with losses varying from 5-50% under field conditions (Kumar and Kumar 2013). However, *F. oxysporum*, *R. stolonifer* and *R. oryzae* were associated with the spoilage of pepper (Tano et al. 2014).

This research work was carried out to identify the fungal species causing post-harvest decay of tomato, pepper and eggplant in major markets in Ilorin, Nigeria and to determine their pathogenicity of each isolate and their preference to carbon nutrients for growth. The outcome of this study would provide quality information on associated fungi and give a clue on the impacts of carbon sources on the growth of fungi isolated from the above-named solanaceous fruits to reduce the losses caused by these fungi.

2. Materials and Methods

2.1. Collection of fruit samples

Healthy and spoilt fruits of *Solanum lycopersicum* (Hausa variety), *Capsicum annuum* (Tatase variety) and *Solanum melongena* (White variety) samples were bought from four major markets in Ilorin, Kwara State, Nigeria viz; "Oja Oba", "Oja tuntun", "Oja Ago" and "Oja Ipata". They were kept in sterilized polythene bags with each bag properly labelled. The samples were then taken to the Plant Biology Laboratory, University of Ilorin for processing.

2.2. Culture media preparation, fungal isolation and identification

For the preparation of the culture medium, 19.5g of Potato Dextrose Agar (PDA) and 32.0g of Sabouraud Dextrose Agar (SDA) were weighed separately and each transferred aseptically using a clean piece of aluminium foil into a sterile 500ml conical flask. It was then made up to the 500ml graduation mark with distilled water. The resulting solution was boiled using hot plate to remove precipitates and shaken intermittently in the case of PDA but SDA was stirred occasionally. After this, each medium was autoclaved at 121^{o}C for 15minutes and 0.1% w/v streptomycin was added to inhibit bacterial growth. Fruits of Tomato, Eggplant and Pepper exhibiting symptoms of spoilage were used for isolation of fungi responsible for the spoilage. Samples from each of the fruits were rinsed with sterile distilled water, surface sterilized with 70% ethanol and rinsed again with sterile distilled water. A sterile scalpel was used to cut 3mm×3mm sections of tissue from the infectious portions of tomato, eggplant and pepper and these portions were aseptically placed on PDA and SDA in Petri dishes. Six replicates were made and they were incubated for five days at room temperature of 25 ± 3°C. After five days, a portion of each colony was picked with a sterilized needle and put into fresh disposable petri dishes with PDA under aseptic conditions to get the pure culture. For identification, the wet mounts of the isolates in lacto-phenol cotton blue were examined using a binocular compound Olympus microscope model (CH) and their mycelial structure, nature of spores and colonial morphology were observed. Identifications were made with reference to Barnett and Hunter (2010) and Campell and Stewart (1980).

2.3. Pathogenicity test

The procedures described by Agrios (2005) and Amadi et al. (2014) were used to test the pathogenicity of each isolate on the selected fruits used in this study. Twelve (12) healthy samples of each fruit were surface sterilized with 70% ethanol and wounded with a sterile scalpel. A 5 mm disc from the pure culture of each fungal isolate was then aseptically transferred into the fruit and this was sealed with Vaseline to prevent the entry of other micro-organisms. The set up was incubated at room temperature of 25 ± 3°C for seven days. The rot diameter of each inoculated fruits was measured with calibrated ruler and recorded.

2.4. Physiological studies of fungal isolates

The method described by Suleiman and Akaajime (2010) was used in this study. The basal medium used to assess the preference of the fungal isolates for carbon nutrients consisted of 1.0g KCl, 0.5g $MgSO_4.7H_2O$, 3.0g $Ca(NO_3)_2$, 1.0g K_2HPO_4 and 0.01g $FeSO_4.7H_2O$. Three carbon sources (glucose, fructose and sucrose) were used to supplement the medium. Each carbon source was dissolved in sterile distilled water in ratio 1:10. Ten milliliter of each solution was aseptically dispensed to 20ml of basal medium in sterile 250ml conical flasks. The flasks were inoculated with 5mm diameter disc of seven-day old culture of each fungal isolate grown on

PDA and then incubated at room temperature for 7 days. Three replicates flasks were used. After 7 days of growth, the mycelia were filtered and dried in an oven at 80^{o}C to a constant weight on pre-weighed Whatman's filter paper. The weights of the mycelia were determined by subtracting the initial weight of the filter paper from the weight of mycelia and filter paper.

3. Results

3.1. Fungal isolation and identification

Aspergillus terreus, Colletotrichum coccodes and *Rhizopus stolonifer* were isolated from tomato fruits while *A. oryzae, Colletotrichum coccodes, Colletotrichum capsici* and *Penicillium funiculosum* were associated with diseased pepper fruits. In the spoilt fruits of Eggplant, *Penicillium funiculosum, Colletotrichum coccodes* and *Schizosaccharomyces pombe* were isolated as shown in Table 1. Only *C. coccodes* was common to the three fruits used in this study.

Table 1: Fungi Isolated from the Selected Solanaceous Fruits.

Fungal isolates	*Tomato*	*Pepper*	*Eggplant*
Aspergillus oryzae	-	+	-
Aspergillus terreus	+	-	-
Colletotrichum coccodes	+	+	+
Colletotrichum capsici	-	+	-
Rhizopus stolonifer	+	-	-
Penicillium funiculosum	-	+	+
Schizosaccharomyces pombe	-	-	+

+ = Present; - = Absent

3.2. Pathogenicity test

The results of the pathogenicity tests showed that all the isolated fungi were pathogenic on each of the solanaceous fruits (Figure 1-3). In tomato, *Colletotrichum coccodes* initiated disease with diameter rot of 3.43cm which was significantly different from rot caused by *Aspergillus terreus* (2.40cm) and *Rhizopus stolonifer* (3.93cm) as shown in Figure 1. *Colletotrichum capsici* caused highest diameter rot in *Capsicum annuum* (4.87 cm) and *Shizosaccharomyces pombe* in eggplant (4.10 cm) as shown in Figures 2 and 3 respectively.

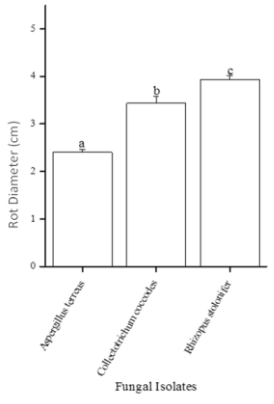

Figure 1: The pathogenicity test of fungal isolates on tomato fruits.

2.5. Data analysis

The data were analyzed using Statistical Package for the Social Sciences (SPPS). The means were separated using Duncan's Multiple Range Test (DMRT).

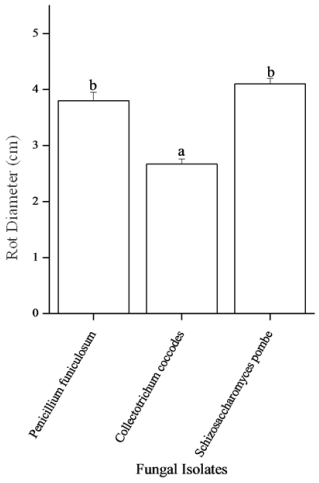

Figure 2: The pathogenicity test of fungal isolates on pepper fruits.

Pathogenicity test revealed that all fungal isolates are pathogenic to their respective host fruit

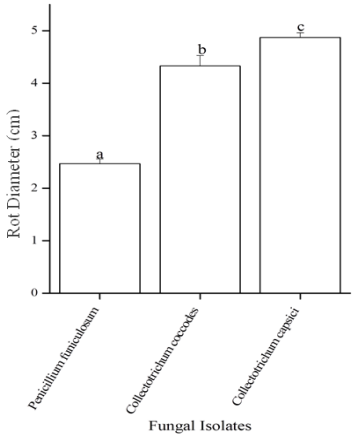

Figure 3: The pathogenicity test of fungal isolates on pepper fruits.

3.3. Physiological studies of the isolated fungi

The physiological studies revealed that the three carbon-source compounds supported the growth of the

fungi isolated in this work. The growths of the two *Aspergillus* species were supported maximally by different carbon sources. Glucose aided the growth of *Aspergillus terreus* with mycelia dry weight of 1.15g which was significantly different from the values recorded for sucrose (1.08g) and fructose (1.00g) as shown in Table 2. *Aspergillus oryzae* grew maximally in sucrose medium (1.10g) (Table 2). Similarly, the mycelial dry weight of both *Colletotrichum coccodes* and *C. capsici* were observed to be highest in sucrose medium (Table 2). In *Penicillum funiculosum, Rhizopus stolonifer* and *Schizosaccharomyces pombe* fructose supported the highest mycelial dry weights. The highest mycelia dry weight of 1.18g recorded in fructose showed that *P. funiculosum* utilized fructose better than other carbon sources, followed by sucrose (1.09g), and glucose had the least mean mycelial dry weight (1.01g) as shown in Table 2. The average mycelia dry weight for *Rhizopus stolonifer* and *Schizosaccharomyces pombe* in fructose medium were1.19g and 1.12g respectively.

Table 2: Mycelial mean dry weight of fungal isolates on different carbon sources

	Aspergillus terreus	***A. oyzae***	***Colletotrichum coccodes***	***C. capsici***	***Penicillum funiculosum***	***Schizosaccharomyces pombe***	***Rhizopus stolonifer***
Carbon source	Mycelia dry weight (g)						
Glucose	1.15 ± 0.04^{a}	0.99 ± 0.00^{a}	1.04 ± 0.01^{b}	0.92 ± 0.02^{b}	1.01 ± 0.03^{b}	0.97 ± 0.02^{b}	1.13 ± 0.01^{b}
Sucrose	1.08 ± 0.01^{b}	1.10 ± 0.01^{a}	1.16 ± 0.03^{a}	1.17 ± 0.01^{a}	1.09 ± 0.00^{b}	1.09 ± 0.04^{a}	1.01 ± 0.01^{c}
Fructose	1.00 ± 0.02^{c}	0.94 ± 0.02^{b}	1.13 ± 0.03^{ab}	0.96 ± 0.01^{b}	1.18 ± 0.04^{a}	1.12 ± 0.01^{a}	1.19 ± 0.01^{a}

Means represent by the same letter(s) along the column are not significantly different at $p \leq 0.05$

4. Discussion

The results obtained in this study is supported by that of Bose et al. (2002) that post-harvest rots of *Capsicum annuum* had been found to be associated mostly with *Aspergillus* spp. and *Colletotrichum* spp. causing severe reduction on the yield and marketability of *Capsicum annuum.* These fungal pathogens might have infected the fruits initially from the field or during storage. *Colletotrichum coccodes* as a common fungal pathogen isolated from the three fruits in this study have been reported to cause anthracnose diseases in tomato, pepper and eggplant fruits which affect not only the quantity but also quality of the fruits (Melanie et al. 2014).

Also, the spores of the isolated fungi might have been dispersed by either animate or inanimate agents. Chuku (2007) reported that flies were carriers of the sporangiospores of *Rhizopus* sp. Since the fungus enters through the broken skin of the fruits, infection usually starts in opening of one type or another. *Rhizopus* soft rot caused by *R. stolonifer* which was isolated only from Tomato fruit in this study is usually spread by *Drosophila melanogaster* which lays its eggs in the growth cracks of various fruits and vegetables. Kobina and Ebenizer (2012) reported that *Aspergillus* spp. and *Penicillium* spp., among other fungal species, were responsible for post-harvest rot of *Capsicum* species. *Aspergillus* and *Penicillium* species are known to produce mycotoxins, which are secondary metabolites of fungi and are cytotoxic with the ability to disrupt cellular structures and interfere with normal biochemical processes of the host (Lillard-Roberts 2014). Carbohydrates play an importance role in biosynthetic and other metabolic activities of organisms. Organic compounds are rich in carbon and support the growth of fungi in varying degree. Glucose supported the growth of *R. stolonifer* and this agreed with the findings of Ibrahim and Shehu (2015). *Aspergillus terreus* grew best in glucose but retarded the growth of *A. oryzae*. Sulaiman and Akaajima (2010) reported that fructose supported the highest mycelial dry weight while sporangia production was more in glucose. The fungi that recorded optimal growth in any carbon source compound possess important growth factors for assimilation of nutrient in the medium and have ability to produce certain hydrolytic enzymes to disintegrate the compound into its units (Ibrahim and Shehu 2015).

5. Conclusion

This study identified the fungi associated with selected solanaceous fruits and showed their levels of pathogenicity. The carbon sources supported the growth of all fungal isolates at different rates. This provides baseline information on the physiological studies of the fungi associated with some selected solanaceous fruits in Ilorin, Nigeria and these can assist to assess the post-harvest losses of the selected fruits.

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