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First Report of *Curvularia spicifera*, As New Causal Agent of Root Rot in Citrus Rootstock (*Citrus aurantium*) in Morocco

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Abstract An isolate of *Curvularia spicifera* obtained from citrus auruntium, was capable of inducing different symptoms in plants of Citrus aurantium, the most widely used citrus rootstock in Morocco. The pathogenicity test was confirmed through dipping inoculation method of roots of Citrus aurantium plants into spore suspension (106 spores/mL) of *Curvularia spicifera* isolate which has led to diverse disease symptoms expression. Among these, root necrosis is accompanied by moderate to general stunting of root system and aerial plant parts. In terms of plant growth parameters, the length of the root and aerial parts were reduced to 6.1 and 6.8 cm in inoculated plants relative to 9 and 12.1 in control plants. The infection was established through the roots which failed to branch normally displaying less density of secondary and tertiary roots with 8/23 compared to 18/42 in the controls. Furthermore, Koch's postulate was verified by re-isolating the original *B. spicifera* isolate from roots of diseased citrus plants. Based on macroscopic, microscopic and pathogenicity characteristics, *Curvularia spicifera* was recognized as a new pathogen of Citrus aurantium rootstock in Morocco.

Keywords *Curvularia spicifera*, *Citrus aurantium*, Pathogenicity, Stunting Symptom

1. Introduction

Citrus is one of the pillars of Moroccan agriculture. It plays an important role in the socio-economic growth on both regional and national scales. It guarantees more than 120.000 stable jobs per year. The annual production reached a volume of 2,3 million tons, exports 650.000 tons with a turnover estimated at 3 billion of dirhams [1]. Morocco is ranked 31 with 203,680 Hectograms per Hectare in 2019 overtaken by Cyprus [2].

Mediterranean basin dominates global citrus exports with 52% of the overall volume, South Africa holds 20%, Asia 13% and South America with 11%. In 2021-2022 season, Morocco's citrus exports attained a record volume of 766500 tone [3] while Spain topped the ranking with 3.5 million tons marketed volume. In Morocco, as is the case in many citrus-growing countries, the majority of the citrus-producing areas are subjected to dry climate and limited availability of water resources with parallel

low-quality irrigation water due to increased salinity regimes [4]. These factors unfavorably impacted citrus tree productivity and fruit quality.

Therefore, extensive work has been conducted upon citrus trees showing that rootstocks are a key component to the ability of the tree to withstand water deficit since they modulate the physiological performance of the tree [5]. Indeed, rootstocks are very important for adaptability to pedo-climatic conditions as well as for tolerance to major biotic and abiotic stresses like *Citrus aurantium* L. [6].

In the context of highly competitive markets, a number of factors such as technical management, pedoclimatic conditions, and rootstock traits, are partially involved in the significant discrepancy in citrus fruit yield among the leading producing countries [6,7,8]. Moreover, citrus crops are vulnerable to many threats and fungal attacks that constitute a serious phytosanitary challenge facing citrus fruit production [9,10]. Among the most widespread citrus diseases in Morocco are citrus phytophthora gummosis caused by *Phytophthora* spp., citrus Phytophthora root rot, anthracnose, and citrus brown rot [7,11,12]. Lahlali et al. [13] have reported Phytophthora rot and dry root rot as highly destructive fungal diseases infecting citrus production. Four Fusarium species have been deemed to be the causal agents of dry root rot of citrus associated with the rapid decline in citrus trees [14]. The use of healthy and resistant rootstocks is an ideal and long-term solution to combating *Phytophthora* spp. among the main soilborne pathogens affecting citrus production worldwide or also *Fusarium* sp. involved in the emerging threat to citrus trees in Morocco [14,15]. However, citrus growers pay a little attention to the mycoflora associated with commercial citrus rootstocks.

The present work aimed to emphasize the ability of one fungal species collected from infected roots of *Citrus aurantium* rootstock to induce root rot symptoms under laboratory conditions.

2. Material and Methods

Fungal Material

In this study, a fungal complex isolated from *Citrus aurantium* rootstocks during a prospecting survey carried out between 2020 and 2021 in citrus orchads of Gharb region (northwest of Morocco) revealed the presence of one isolate of *Curvularia* sp. sourced from root parts.

Morphological, Cultural and Molecular Characteristics of *Curvularia* sp. Isolate

Single spores of *Curvularia* sp. isolate were transferred to potato-sucrose agar (PSA) (200g of potato, 15g of agar, 20g of sucrose and 1000 mL of distilled water) and incubated at room temperature (28 °C) for 7 days. After one week of incubation, the morphological characters were recorded (colony aspect and color, mycelial density, conidia and conidiophore shape, color and dimension). Morphological identification was achieved by observing the fungus colony and conidia based on characteristics described by Ellis [16] and earlier studies for *Curvularia* genus [17,18,19,20,21]. Molecular characterization was performed using genomic DNA extraction from the original isolate. Ribosomal DNA ITS and 28S regions were amplified with universal primer pairs ITS1/ITS4 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) and Sequencing was performed on an ABI 3130XL Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA).

Pathogenicity Test

Plant Material

The pathogenicity test was performed with seed sowing. *Citrus aurantium* L. seeds were surface sterilized with 2% sodium hypochlorite ($NaClO_2$) for three minutes, washed three times with sterile distilled water and dried on sterile blotter paper for two minutes then sown in plastic pots filled with a mixture of peat and Maamora soil disinfected in an oven at 250°C for 2 hours. Pots were kept under greenhouse conditions and watered daily with tap water until they reached 2-leaves stage (35 days after sowing of citrus seeds).

Inoculum Preparation

Inoculum suspensions were prepared from fresh, mature fungal cultures of *Curvularia* sp. grown onto Petri- dishes containing PSA medium at 28°C for 15 days. The colonies were covered with 5 ml of distilled sterile water. Tween 20 (5%) was added to facilitate the preparation of Curvularia inoculum which was achieved by carefully rubbing the colonies with a sterile loop; the suspension was filtered and collected in a sterile tube. Then the suspension was adjusted to a final concentration of 106 conidia/mL using the Malassez (Precicolor HBG in Germany)

Inoculation

After 35 days, the seedlings (2–3 leaf stage) were carefully uprooted from the potting substrate (peat and Maamora soil), and the root system was washed with tap water to remove the peat and soil particles. The roots of the seedlings were immersed in the 180 ml of inoculum suspension freshly prepared for 30 minutes. The inoculated seedlings were individually transplanted into (1 seedling/ pot) and subsequent addition of 10 mL of conidial suspension (1×10^6 conidia mL^{-1}) to each pot. In the control treatment, roots were soaked in distilled sterile water. Seedlings were watered with tap water at two days' interval.

Disease Symptom Assessment

Growth Parameters

The growth response of citrus seedlings was assessed 7 days after inoculation: length of aerial parts and below-ground parts; fresh and dry weight of aerial and root parts; number of leaves; secondary and tertiary root development. The fungus was re-isolated in PSA medium and compared against the original strains. Control plants were sprayed with sterilized distilled water.

Re-isolation of Causal Agent

Re-isolations were established from the seedlings showing growth disorders and weak development. The fungus was re-isolated from citrus plants 45 days after inoculation in PSA medium and compared against the original strains. Control plants were sprayed with sterilized distilled water.

The inoculated plants were uprooted, and cleaned of soil debris, then washed thoroughly with running water. The roots were cut out into small pieces of 2–3 mm and then immersed in 1% sodium hypochlorite (NaOCl) for 1–2 min, rinsed thereafter three times with sterilized water, dried on sterile filter paper and placed in Petri dishes containing three discs of Wattman paper soaked with sterile distilled water. The different wet chambers were placed under continuous light at 28°C in order to promote sporulation of the fungus. After several days of incubation, root segments were examined under a light microscope under aseptic conditions. Hence, the presence or absence of conidia of *Curvularia spicifera* was recorded.

Four replicates of four citrus seedlings were used for the pathogenicity test of the *C. spicifera* isolate studied as control plants.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and LSD test at 5% level.

3. Results and Discussion

The fungal isolate of *Curvularia* sp. isolated from necrotic lesions as external symptoms on the roots of *Citrus aurantium* has been grown onto PSA medium, on which a dark greyish colony was developed 7 days later (Figure 1A). The mycelium of this isolate was septate, and conidiophores were pale brown, reddish brown to dark brown, septate, solitary or grouped, and geniculate several times (Figure 1B). Conidia are straight, cylindrical, rounded at the ends, formed by 3 cells of golden-brown color, constantly smooth and measuring from 23 to 30 µm × 10 to 13 µm (Figure 1C). Intercalary chlamydospores appeared as the culture aged. This description matched with that given by many research works [20,21,22] to *Curvularia spicifera*. Basic Local Alignment Search Tool (BLAST) analysis of ITS sequences indicated that the isolate (GenBank Accession No OM980225) showed 100% identity with *C. spicifera.*

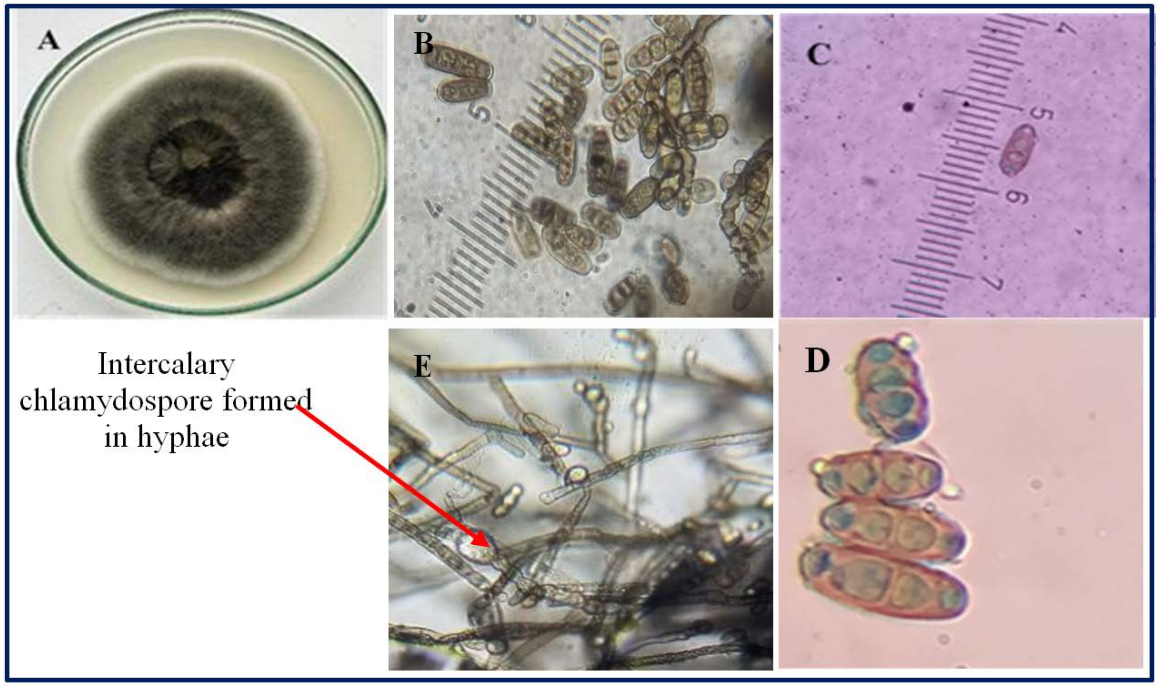

Figure 1. Microscopic characterization of the Curvularia spicifera isolate. Different components of C. spicifera life cycle: colony onto PSA medium (A); conidiophores and conidia (B, C, D); intercalary chlamydospores formed in hyphae (E)

Following inoculation with *Curvularia spicifera*, *Citrus aurantium* plants developed external symptoms such as root necrosis and plant stunting (Figure 2B).

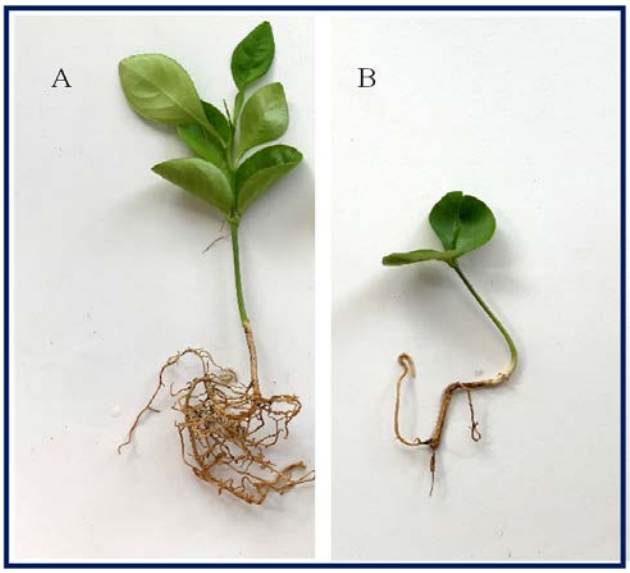

Figure 2. Plants of *Citrus aurantium* 45 days after inoculation: (A) Control plants; (B) Inoculated plants by *Curvularia spicifera*

Curvularia spicifera isolate had strongly affected root system and stem growth. 45 days after transplanting (Figure 3A, B). The average lengths of the root and aerial parts of inoculated plants were 5.7 and 12.1 cm, respectively, compared to those of control plants 4.1/6.8 cm. The fresh weights of the roots and the vegetative part were respectively, 0.09 g and 0.2 g for the inoculated plants and 0.3 g and 0.5 g for the control ones (Table 1).

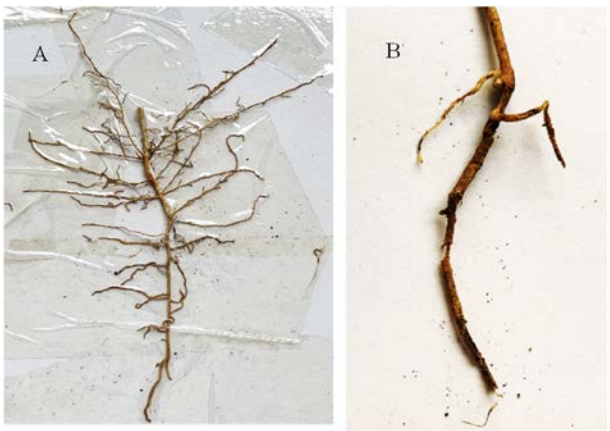

Figure 3. Root system of inoculated *Citrus aurantium* plants 45 days after transplanting. (A) Root of control plant; (B) Root of inoculted plant by *Curvularia spicifera* isolate

The alteration of root structure was focused on the inhibition of primary and lateral root elongation and density (Figure 3A, B). The average number of secondary and tertiary roots was 18/ 2 in inoculated plants compared to 42/4. It has been observed that the bark of the main root was detached. In addition, a decreasing mean number of leaves was noted (Table 2). The average number of leaves recorded in inoculated and control plants after 45 days of cultivation was 3 and 6, respectively (Table 2).

Table 1. Effect of *C. spicifera* on growth parameters of inoculated *Citrus aurantium* plants

	Biomass (g)		Length (cm)	
	Aerial part	Root parts	Aerial parts	Root parts
Control plants	0.5[a]	0.3[a]	6.8[a]	12.1[a]
Inoculated plants	0.2[b]	0.09[b]	4.1[c]	5.7[c]

* The values in columns with minuscules indicate a statistical difference ($P < 0.05$).

Table 2. Number of secondary and tertiary roots, number of leaves in inoculated and un-inoculated citrus plants by *Curvularia spicifera*

	Number of leaves	Number of secondary roots	Number of tertiary roots
Control seedlings	6[a]	18[a]	42[a]
Inoculated Citrus seedlings	3[b]	2[c]	4[c]

* The values in column with minuscules indicate statistical difference ($P < 0.05$)

The re-isolation from the roots of inoculated plants after observation of symptoms was performed, and isolates were compared to the original culture, providing evidence for fulfilling Koch's postulates.

The isolate of Curvularia *spicifera* produced the sympodial conidiophore and had typical traits of the genus which matched with those described by many authors [17,18,19,20,21,23]. In several taxonomic refinements, due to morphological attribute similarities, Bipolaris and Curvularia are included in Helminthosporium species and recognized as helminthosporioid fungi [24,25]. Many species of this genus are known to infect a wide range of hosts around the world causing leaf spot, root rot and seedling blight. Qostal et al. [21] have reported *C. spicifera* as phytopathogenic in wheat and barley varieties, able to reproduce necrotic lesions on the roots and leaves of seedlings. It is also responsible for leaf spots on *Punica* [26], the ornamental plant *Erythrina caffra* [27], *Citrillus lunatus* [15] and *Ficus retusa* nitida [16]. In agreement with our findings, Jamali et al. [28] have demonstrated the root system attack of *Pyrus communis* by *Curvularia inequalis*, which also developed foliar yellowing and black cankers that extended upward and downward of roots from all emerged seedlings. During transmission studies, Gupta et al. [29] found that *Curvularia lunata* moved from seed to seedling/plant which caused high pre- and post-emergence losses, and symptoms such as brown necrotic spots on primary leaves and later the whole seedling got rotten, subsequently succumbing to death. In

Azerbaijan, *C. spicifera* occurred among fungal complexes associated with root and crown rot of wheat and was confirmed to be one the causal agents [30].

4. Conclusions

The results of our study confirm a characteristic of symptoms affecting below-ground parts of citrus seedling, which resulted in a decrease in plant growth parameters and whole plant stunting. To the best of our knowledge, this is the first report of *Curvularia spicifera* as a fungal pathogen causing root rot of *Citrus aurantium* rootstock the most commonly used citrus rootstock in Morocco [31].

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REFERENCES

[1] Oubelkacem A. Scardigno A., Choukr Allah R., "Treated Wastewater Reuse on Citrus in Morocco: Assessing the Economic Feasibility of Irrigation and Nutrient Management Strategies", Integrated Environmental Assessment and Management, vol. 16, 6, pp. 898–909, 2020, https://doi.org/10.1002/ieam.4314

[2] FAOSTAT, 2019. Citrus Yield in Morocco (nationmaster.com)

[3] Anonymous, 2022. Morocco's Citrus Exports Reach 766,500 Tonnes in 2021-2022 Season. https://www.moroccoworldnews.com/2022/09/351236.

[4] Syvertsen J.P., "Garcia-Sanchez, F. Multiple abiotic stresses occurring with salinity stress in citrus". Environ. Exp. Bot. 103, pp.128–137, 2014, https://api.semanticscholar.org/CorpusID:84703339

[5] Romero P., Navarro J.M., Pérez-Pérez J., García-Sánchez F., Gómez-Gómez A., Porra I., Martinez V., Botía P., "Deficit irrigation and rootstock: Their effects on water relations, vegetative development, yield, fruit quality and mineral nutrition of Clemenules mandarin". Tree Physiol., *26*, pp. 1537–1548, 2006, DOI: 10.1093/treephys/26.12.1537

[6] Benyahia H., "Amélioration de la résistance des porte-greffes d'agrumes vis-à vis des contraintes biotiques et abiotiques", Faculté des Sciences Dhar El Mahraz - Fès - Doctorat ou Doctorat National. 2007.

[7] Benyahia H. Talha A., Fadli A., Chetto O., Omari F. E., Beniken L., "Performance of 'Valencia Late' Sweet Orange (Citrus sinensis) on different rootstocks in the Gharb Region (Northwestern Morocco)", Annual Research & Review in Biology, vol. 20, 4, pp. 1-11, 2017, DOI: 10.9734/ARRB/2017/37924

[8] Kunta M., Chavez S., Viloria Z., del Rio H. S., Devanaboina M., Yanev G., Park J-W., Eliezer S., Louzada E. S., "Screening Potential Citrus Rootstocks for *Phytophthora nicotianae* tolerance", Hortscience, vol. 55 (7), pp. 1038–1044, 2020, https://doi.org/10.21273/HORTSCI14920-20

[9] Khanchouch K., Pane A., Ali Chriki A., Cacciola S. O., "Major and Emerging Fungal Diseases of Citrus in the Mediterranean Region", IntechOpen, 2017, DOI: 10.5772/66943

[10] Lacirignola C., D'Onghia A. M., 2009. The Mediterranean citriculture: productions and perspectives. In: D'Onghia A.M. (ed.), Djelouah K. (ed.), Roistacher C.N. (ed.), "Citrus tristeza virus and Toxopteracitricidus: a serious threat to the Mediterranean citrus industry", Bari: CIHEAM, pp. 13-17, 2009, http://om.ciheam.org/article.php?IDPDF=801383

[11] Benyahia H., Jrifi A., Smaili C., Afellah M., Lamsetef Y., Timmer L.W., "First report of *Colletotrichum gloeosporioides* causing wither-tip on Twigs and Tear Stain on Fruit of Citrus in Morocco", Plant Pathology, vol. 52, p. 798. 2003, https://doi.org/10.1111/j.1365- 3059.2003.00907.x

[12] Jaouad M., Moinina A., Ezrari S., Lahlali R., "Key pests and diseases of citrus trees with emphasis on root rot diseases: An overview", Mor. J. Agri. Sci. vol. 1(3), pp. 149-160, 2020, https://api.semanticscholar.org/CorpusID:225887968

[13] Lahlali R., Jaouad M., Moinina A., Mokrini F., Belabess Z., "Farmers' knowledge, perceptions, and farm-level management practices of citrus pests and diseases in Morocco". Journal of Plant Diseases and Protection, Vol. 128, pp. 1213-1226, 2021 https://doi.org/10.1007/s41348-021-00479-2

[14] Ezrari S., Lahlali R., Radouane N., Tahiri A., Asfers A., Boughalleb-M'Hamdi N., Amiri S., Lazraq A., "Characterization of Fusarium species causing dry root rot disease of citrus trees in Morocco", J Plant Dis Prot, vol. 128, pp. 431–447, 2021,https://doi.org/10.1007/s41348-020-00392-0

[15] Chaudhary S., Laughlin D. A., Setamou M., da Graça J. V., Kunta M., Alabi O. J., Crosby K. M., Ong K. L., Ancona V., "Incidence, severity, and characterization of Phytophthora foot rot of citrus in Texas and implications for disease management", Plant Disease, vol. 104, pp. 2455-2461, 2020, https://doi.org/10.1094/PDIS-07-19-1493-RE

[16] Ellis D. E., Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, 608 pages. 1971.

[17] El Mhadri M., Benkirane R., Ouazzani Touhami A., Douira A., "*Citrullus lanatus*, a new host of Bipolaris spicifera in Morocco", Phytopathologia Mediterranea, vol. 48, pp. 291-293, 2009, https://www.jstor.org/stable/26463354

[18] Kadri O., Benkirane R., Ouazzani Touhami A., and Douira A., "Punica granatum: A New Host of *Bipolaris spicifera* in Morocco", Atlas Journal of Biology, 1 (3): pp. 47-51, 2011

[19] Kachkouch W., Ouazzani Touhami A., Benkirane R.,

Douira A., "Bipolaris spicifera, un nouveau parasite foliaire de l'espèce ornementale, Erythrinacaffra, au Maroc", Int. J. Biol. Chem. Sci. Vol. 5 (6), pp. 2491-2496. 2011, DOI: 10.4314/ijbcs.v5i6.27

[20] Drider R., Ouazzani Touhami A., Hsissou D., Benkirane R., Douira A., "*Bipolaris spicifera* a new foliar parasite of Ficus retusa nitida in Morocco", Bulletin de la Société Royale des Sciences de Liège, vol. 80, pp. 1-7, 2011. URL: https://popups.uliege.be/0037-9565/index.php?id=3336.

[21] Qostal S., Kribel S., Chliyeh M., Selmaoui K., Ouazzani Touhami A., Serghat S., Zaarati H., Benkirane R., Douira A., "*Curvularia spicifera*, a parasite of the fungal complex of root rot of wheat and barley in Morocco", Plant Cell Biotechnology and Molecular Biology, vol. 20, (9&10), pp. 354-365, 2019, https://www.ikprress.org/index.php/PCBMB/article/view/4627

[22] Amaradasa B. S., Amundsen K., "First Report of *Curvularia inaequalis* and *Bipolaris spicifera* Causing Leaf Blight of Buffalograss in Nebraska". Plant Disease, vol. 98(2), pp. 279, 2014, https://doi.org/10.1094/PDIS-05-13-0487-PDN

[23] Jeon S. J., Nguyen T. T. T., Lee H. B., "Phylogenetic status of an unrecorded species of Curvularia, *C. spicifera*, based on current classification system of Curvularia and Bipolaris group using Multi Loci", Mycobiology vol. 43(3), pp. 210-217, 2015, http://dx.doi.org/10.5941/MYCO.2015.43.3.2102015

[24] Ünal F., Turgay E. B., Yıldırım A. F., Yüksel C., "First report of leaf blotch on sorghum caused by *Bipolaris spicifera* in Turkey". Plant Disease, vol. 95 (4), p. 495, 2011, DOI: 10.1094/PDIS-01-10-0037

[25] Sivanesan A., "Graminicolous species of Bipolaris, Curvularia, Drechslera, Exserohilum and their teleomorphs". Mycological Papers No. 158; C.A.B. International: Wallingford, UK, pp. 1–261, 1987.

[26] Manamgoda D. S., Cai L., Bahkali A. H., Chukeatirote E., Hyde K. D., "Cochliobolus: An overview and current status of species", Fungal Divers. vol. 51, pp. 3–42, 2011, https://doi.org/10.1007/s13225-011-0139-4

[27] Kadri O., Benkirane R., Ouazzani Touhami A., and Allal Douira A., "*Punica granatum:* A New Host of *Bipolaris spicifera* in Morocco", Atlas Journal of Biology, vol. 1 (3), pp. 47-51, 2011, DOI: 10.5147/ajb.2011.0047

[28] Jamali S., Khateri H., Rezaei N., "Black root rot caused by *Curvularia inaequalis* on *Pyrus communis* in Iran", Australasian Plant Disease Notes. Vol. 15, p. 22, 2020, DOI: https://doi.org/10.1007/s13314-020-00388-7

[29] Gupta S., Dubey A., Singh T., "Curvularia lunata as, a dominant seed-borne pathogen in Dalbergia sissoo Roxb: Its location in seed and its phytopathological effects", African Journal of Plant Science, vol. 11(6), pp. 203-208, 2017, DOI: 10.5897/AJPS2017.1529

[30] Özer G., Paulitz T. C., Imren M., Alkan M., Muminjanov H., Dababat A. A. "Identity and pathogenicity of fungi associated with crown and root rot of dryland winter wheat in Azerbaijan", Plant Disease, vol. 104, pp. 2149-2157, 2020, https://doi.org/10.1094/PDIS-08-19-1799-RE

[31] El-Otmani M., Coggins, Jr. C. W., Duymovic A., "Citrus Cultivars and Production in Morocco". HORTSCIENCE, vol. 25(11), pp. 1343-1346, 1990, DOI: 10.21273/HORTSCI.25.11.1343