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Curvularia spicifera, A PARASITE OF THE FUNGAL COMPLEX OF ROOT ROT OF WHEAT AND BARLEY IN MOROCCO

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ABSTRACT

Surveys were carried out in wheat (durum wheat and soft wheat) and barley fields in April-May 2017 in northwestern Morocco allowed the isolation of a species of the *Curvularia* genus from the characteristic necrotic lesions of the root rot of wheat and barley. This fungus was isolated either in the presence of *Bipolaris sorokiniana* or among a variable Fusarium complex. It has been identified as *Curvularia spicifera*. Koch's postulate was verified by inoculating six isolates of this fungus into varieties of wheat (durum wheat and soft wheat) and barley. All of these isolates induced necrotic lesions on the roots and leaves of seedlings from the inoculated grain. Re-isolations of the pathogen from these lesions were positive. Severity indices can reach 90% in hard and soft wheat and 88% in barley, with incidences of the disease ranging from 50% to 96%. Sporulation at the root and leaf lesions of wheat plants is important; it varies between 6.2 10^6 and 1.9 10^6 conidia / cm^2 in durum wheat and between 6.11 10^6 and 4.88 10^6 conidia / cm^2 in common wheat. On the other hand, it varies between 5.8 10^6 and 0.93 10^6 conidia / cm^2 in barley. To our knowledge, this is the first report of root rot caused by *Curvularia spicifera* on wheat and barley in Morocco. The pathogen will probably become important and take up more of the other known diseases of cereals.

Keywords: Wheat; barley; roots; lesions; *Curvularia spicifera*; Koch postulate.

INTRODUCTION

Curvularia spicifera (Bainier) Boedijn, 1933 (synonym *Helminthosporium spiciferum* (Bainier) Nicot and *Bipolaris spicifera)* [1], filamentous fungi of the family Pleosporaceae, is the imperfect form of *Cochliobolus spicifera* Nelson [2,3,4]. Although the disease induced by this species has been reported in almost every continent, in America (Mexico, Argentina), Europe (Greece), Asia (India, Irak, Pakistan, China and Iran) and in Africa (Egypt, Morocco) [5,6], its influence on yields remains negligible [7]. According to Behdani et al. [8], *Curvularia spicifera,* in general, has a low virulence, but in case of epidemic and under certain favorable conditions, can cause a loss of yield.

Curvularia spicifera has been reported as a causal agent for *Cynodon* and *Zoysia stem* rot [9], and leaf lesions on the date palm and ornamental palm [10]. It was also isolated from the ground [11] and from the seeds of some herbs [12]. Zillinsky [7] noted that this pathogen occasionally attacks wheat, rice and other cereals, and is usually considered to be a low virulent pathogen that

causes wheat crown rot, but also foliar symptoms of irregular shapes spread throughout the length and width of the leaf blade of soft wheat and durum wheat (in northern india and Pakistan) and barley (in Mexico). *C. spicifera* was isolated in Iran from ears and wheat kernels [13], from roots and barley leaves [5,14] and from foliar lesions of barley [15]. Mehrian et al. [16], considered this fungus as a foliar pathogen of corn and as a causal agent for stem rot of this plant species.

In Morocco, *Curvularia spicifera* was isolated from rice seeds [17] and foliar lesions of rice plants [6,18], of *Hibiscus rosa-sinensis* [19], *Citrullus lanatus* [20], *Punica granatum* [21], *Erythrina caffra* [22], *Ficus retusanitida* [23], *Musa accuminata* [24] some plants of strawberry [25]. All these authors noted that this pathogen was able to induce sporulating foliar lesions on these different plant species after their inoculation and it is also responsible for the tarnishing of rice grains [26].

Surveys carried out in wheat (durum wheat and soft wheat) and barley fields in April-May 2017 in northwestern Morocco have made it possible to isolate from the necrotic lesions of the roots of these species a variable fungal complex composed of *Bipolaris sorokiniana, Curvularia spicifera* (synonym *Helminthosporium spiciferum)* and some Fusarium species such as *Fusarium culmorum*, *F. solani, F. nivale, F. poae, F. oxysporum, F. graminearum, Fusarium* sp. and *F. roseum* [27]. The percentages of isolation of these species vary between 5 and 27%. *Curvularia spicifera* was isolated for the first time in Morocco from the roots of wheat and barley showing the symptoms of decay and the part that belongs to this species in the development of root rot is not known in the literature.

In this work, the ability of *C. spicifera* to induce the symptoms of root rot has been studied in some varieties of wheat (durum and soft) and barley widely grown in Morocco.

MATERIALS AND METHODS

Fungal Material

Six isolates of *Curvularia spicifera*, isolated from the roots of durum wheat, soft wheat and barley plants growing in different areas of northwestern Morocco (Table 1) are preserved on small filter paper washers in freezer filter paper at -20°C.

Table 1. The isolates of *Curvularia spicifera* and their origins

Curvularia spicifera isolate	Isolation sources	Localities
H5	Durum wheat	Megren
H6	Soft wheat	Allal Tazi
H7	Soft wheat	Souk Larbaa
H8	Barley	Ouled Sellam
H9	Barley	Allal Tazi
H10	Barley	Ouazzane

Plant Material

Grains, healthy-looking caryopses, durum wheat (Variety: Amjad), soft wheat (Variety: Amal) and barley (Escourgeons) are superficially disinfected by soaking for 2 min in a solution of sodium hypochlorite. 10% followed by six rinses with sterile distilled water. The grains thus disinfected are allowed to dry on sterile filter paper for 24 hours.

Preparation of the Inoculum

The six isolates of *Curvularia spicifera* are subcultured in 90 mm diameter Petri dishes on PSA medium (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g Agar-agar, 1000 mL distilled water). This agar medium was previously sterilized by autoclaving at 120°C. for 30 minutes. When it cools and reaches a temperature of about 50°C, the medium was poured into Petri dishes at a rate of 30 to 40 mL per dish supplemented with 100 mg/L of chloramphenicol. Incubation of isolate cultures was performed in the dark at 28°C.

After 15 days of incubation, the spore-laden surface is scraped off with a metal spatula in the presence of 10 ml of sterile distilled water. The suspension is then stirred for one minute and then filtered through muslin to separate the spores from the mycelial fragments. After counting with a Malassez slide, the spore suspension is adjusted with sterile distilled water so as to obtain a final concentration of 10^5 spores / ml. The suspension is then added with 0.05% Tween 20 and 0.5% gelatin.

Inoculation of Grains

The disinfected seeds are inoculated by dipping for 24 h in a spore suspension of 10^5 spores /mL of each of the tested isolates, and then dried on sterile filter paper. Control grains are treated only with sterile distilled water.

Inoculated and control seeds are sown in plastic pots (4 grains / pot) containing a sterile mixture of peat (25%) and Mamora sand (75%). After, all the pots are brought back in a culture greenhouse.

The severity of the disease was evaluated at the flowering stage and focused on the description of the attack on the entire root system, namely the snare, the snare and the seminal roots. The expression of the severity of the disease at the level of the root system was evaluated according to a class scale of 0 to 5.

The reisolation of *C. spicifera* was carried out at the ripening stage from the control and inoculated plants. The plants were dug up and cleared of their growing medium by washing them thoroughly with running water. Cross sections of 2 cm, made in the roots and crown were performed. The different levels of sections were separately deposited in the alcohol at 90° for 1 to 2 minutes, rinsed thoroughly with sterile water, dried on a sterile filter paper and deposited on PSA medium.

Re-isolation was also performed from foliar lesions. Fragments of foliar lesions from wheat and barley plants were disinfected as before and incubated on filter paper soaked in sterile water in Petri dishes under white light at 28°C until conidia were obtained. After 7 days of incubation, the cut fragments were examined under an optical microscope under aseptic conditions to detect the presence or absence of the conidia of the fungus. The transfer of conidia was made under a microscope using a drawn glass capillary previously sterilized flame and cooled in the culture medium. The transferred conidia were deposited on the surface of the PSA culture medium.

Sporulation on the Host

Sporulation is measured according to the technique of Nelson [28] by estimating the average number of conidia produced per unit area of the roots and leaves of the host carrying the necrotic lesions (expressed in number of spores / cm^2). Roots and leaves taken from plants with root and leaf symptoms, ripening stage, are cut into 4-5 fragments and then placed in petri dishes containing filter paper soaked in sterile distilled water. The dishes are placed under continuous fluorescent light for 7 to 8 days at 28°C. The fragments are then recovered in test tubes containing 1 mL of sterile distilled water. The tubes are agitated so as to detach the conidia of the mycelium. The richness of the suspensions thus obtained is determined using a Malassez slide and the number of conidia and returned to the unit area of the sheet. The observation is made under an optical microscope (× 100).

Assessment of the Disease Severity

In the laboratory, the root part of the plants was examined visually and the disease was evaluated according to the Greany et al. [29] who distinguished six severity classes according to the types of observed symptoms: S0, no infection; S1, small necrotic lesions dispersed at the sub-crown and root; S2, distinct necrotic lesions on the basal part of the plant, particularly in the sub-collar and roots; S3, large necrotic lesions on the neck, the sub-collar and the roots with decrease of the vigor of the plant; S4, rots of the basal part, chlorosis of the plant, often dwarfing and wilting and S5, dead plant.

The incidence of the disease was calculated according to the following formula: $I = 100\ [Nm / Nt]$, that is to say the percentage of diseased plants, Nm reported as the total number Nt of examined plants. And the root rot index $IM = 100\ \Sigma\ (Ni\ Si) / (5\ Nt)$, with Ni number of plants of severity class i and Si severity class i.

Statistical Analysis

Statistical analyzes were performed by analysis of the variance by the 5% ANOVA test.

RESULTS

From a morphological point of view, the studied isolates of *Curvularia spicifera* presented on PSA

medium a septate mycelium of greyish color, solitary conidiophores or in small groups, with cords, geniculate several times, half-brown to black. Conidia are straight or cylindrical, rounded at the ends. Each conidia is composed of 3 cells of golden brown color, constantly smooth and measure from 23 to 30 µm × 10 to 13 µm (Fig. 3). This description is identical to that given by Ellis [30,20,23,22,31]. Similarly, all isolates of *C. spicifera* have formed resistance elements which are intercalated chlamydospores resulting from transformation of the cells of already formed vegetative hyphae. These chlamydospores, thallospores, are isolated or formed from two, three or more cells, sometimes in chains, between the cells of the mycelial filaments. Chlamydospores developing at the end of the mycelial filaments were also observed. It is important to note that it is for the first time that chlamydospores have been noted in *C. spicifera.*

Artificial inoculation of wheat (durum and soft) and barley grains with tested isolates of *Curvularia spicifera* induced the same symptoms of root rot as observed in the plants of these cereals. The induced symptoms by isolates concerned the roots and also the aerial parts of the plants (Figs. 1 and 2). At the early maturation stage, the size of the necrotic lesions observed on the roots and at the sub-collar of the plants is very variable and depends on the varieties of the host plants and the tested isolates. The noted foliar lesions are early small elongated to elliptic lesions of white color and become, by time, dark brown in the center to bright brown on the periphery. The disease has also led to the appearance of partially or totally scalded ears with grains with variable darkening.

At the early stage of ripening, root rot was observed in a large number of wheat (durum and soft) and barley plants from seed inoculated with the tested *C. spicifera* isolates. In general, a predominance of severity classes of S2 and S3 is observed in durum wheat (vary respectively between 25 and 33.3 and 16.6 and 33.33%) and soft wheat (between 25 and 33.33 and between 16.6 and 33.33, respectively), followed by S4 (range from 8.33 to 16.7%). S1 severity classes vary between 8.33 and 25% in durum wheat, between 0 and 33.33% in common wheat and between 8.33 and 41.66% in barley. The percentage of inoculated plants did not show a symptom of decay (class S0) varies between 16.7 and 25% in durum wheat, from 0 to 16.66% in soft wheat and between 8.33 and 25% in barley (Table 2).

Table 2. Percentage of severity classes of root rot disease on wheat and barley plants

Varieties of wheat and barley	Isolates	Severity class				
		S0	**S1**	**S2**	**S3**	**S4**
Durum wheat 'Amjad'	H5	16.7	16.7	25	25	16.7
	H6	16.6	8.33	33.33	33.33	8.33
	H7	16.6	25	25	16.6	16.6
	H8	16.6	16.6	25	16.6	25
	H9	8,33	25	25	16,6	25
	H10	25	8.33	25	25	16.6
Soft wheat 'Amal'	H5	0	16.6	33.33	33.33	16.6
	H6.	16.6	16.6	33.33	25	8.33
	H7	8.33	33.33	25	16.6	16.6
	H8	0	16.6	33.33	33.33	16.6
	H9	8.33	25	25	16.6	25
	H10	0	16.6	25	25	33.33
Barly 'Escourgeon'	H5	8.33	41.66	16.6	25	8.33
	H6	33.33	25	25	8.33	8.33
	H7	8.33	25	33.33	16.6	16.6
	H8	25	8.33	33.33	25	8.33
	H9	8.33	33.33	33.33	25	0
	H10	25	25	25	16.6	8.33

Isolates of *C. spicifera*, originating in barley, induced the highest percentages of S4 severity class. The isolate H10C Barly Ouazzane induced percentages of the order of 33.33 and 25%, respectively in wheat and durum wheat. This isolate only induced a percentage of 8.33% in barley. The H9C Barley Tazi isolate, originating from barley, induced a percentage of the S4 severity class of about 25% in common and durum wheat, The H9C Barley Tazi isolate, originating from barley, induced a percentage of the S4 severity class of about 25% in common and durum wheat. The lowest percentage (8.33%) was observed in plants grown from durum wheat and soft wheat grains inoculated with H6. Tazi isolate from soft wheat.

On barley, are the isolates from durum wheat (H5C Megren BD) and wheat (H6.BT Tazi and H7.C Larbaa BT) those induce percentages of class S4 higher severity (range between 8.33 and 16.6%) to those originating from barley (H8.C Barly Sellam, H9.C Barly Tazi and H10.C Barly Ouazzane) which vary between 0 and 8.33%.

All the tested *C. spicifera* isolates sporulated on the roots of wheat (durum and soft) and tested barley (Table 3). This sporulation is a function of the studied cereal species. On the roots of durum wheat, soft wheat and barley it is isolate H8. C Barley Sellam, originating from barley, which may have sporulated significantly on the roots of these cereals, respectively 6.37, 6.14 and 5.7310^6 spores / cm2, followed in general by BD Megren isolate H5C from wheat, with respectively a sporulation of 6.2, 6.11 and 5.1 10^6 spores / cm^2. On the other hand, it is the isolate, H10. C. Barley Ouazzane, from barley, which submitted the lowest sporulation on the roots of these cereals, varies between 0.93 (barley) and 3.8810^6 spores / cm^2 (durum).

The incidence and index of the disease vary significantly according to the host species (common wheat, durum wheat, barley) and the tested *C. spicifera* isolate (Table 4). In general, the incidence of the disease is high and varies between 75 and 91.6% in the Amjad variety (durum wheat), between 83.3 and 100% for the Amal variety (soft wheat) and between 66.6 and 91.6% for the barley (Escourgeon variety). H5C.BD Megren isolates, native to durum wheat, and H8. C Barley Sellam, barley, induced in soft wheat an incidence of 100%.

Table 3. Sporulation of *C. spicifera* on the roots of wheat and barley varieties (x 10^6 spores / cm^2)

Varieties of wheat and barley	Isolates	Sporulation (10^6 spores/cm^2)
	H5	6.2^{a}
	H6	5.9^{b}
Durum wheat	H7	4.55^{d}
Amjad	H8	6.37^{a}
	H9	2.8^{f}
	H10	3.88^{e}
	H5	$6.11a^{b}$
	H6	5.1^{c}
Soft wheat	H7	4.37^{de}
Amal	H8	6.14^{a}
	H9	4.88^{c}
	H10	3.33^{e}
	H5	5.1^{c}
	H6	2.68^{f}
Barley	H7	1.60^{g}
Escourgeon	H8	5.73^{b}
	H9	4.8^{c}
	H10	0.93^{h}

Two results read on the same line differ significantly at the 5% threshold if they are not assigned any letter in common

Table 4. Incidence (%) and Root Rot Index (%) for durum wheat, soft wheat and barley

Varieties of wheat and barley	Isolates	Incidence I (%)	Disease index IM (%)
Durum wheat 'Amjad'	H5	83.3^{c}	61.6^{c}
	H6	83.3^{c}	61.6^{c}
	H7	83.3^{c}	70^{b}
	H8	83.3^{c}	58.3^{d}
	H9	91.6^{b}	61.6^{c}
	H10	75^{d}	65^{b}
Soft wheat 'Amal'	H5	100^{a}	70^{b}
	H6	83.3^{c}	66.6^{b}
	H7	91.6^{b}	60^{c}
	H8	100^{a}	66.6^{b}
	H9	91.6^{b}	65^{b}
	H10	83.3^{c}	75^{a}
Barley 'Escourgeon'	H5	91.6^{b}	43.3^{f}
	H6	66.6^{e}	51.66^{e}
	H7	91.6^{b}	61.6^{c}
	H8	75^{d}	61.6^{c}
	H9	91.6^{b}	55d^{e}
	H10	75^{d}	51.6^{e}

Two results read on the same line differ significantly at the 5% threshold if they are not assigned any letter in common

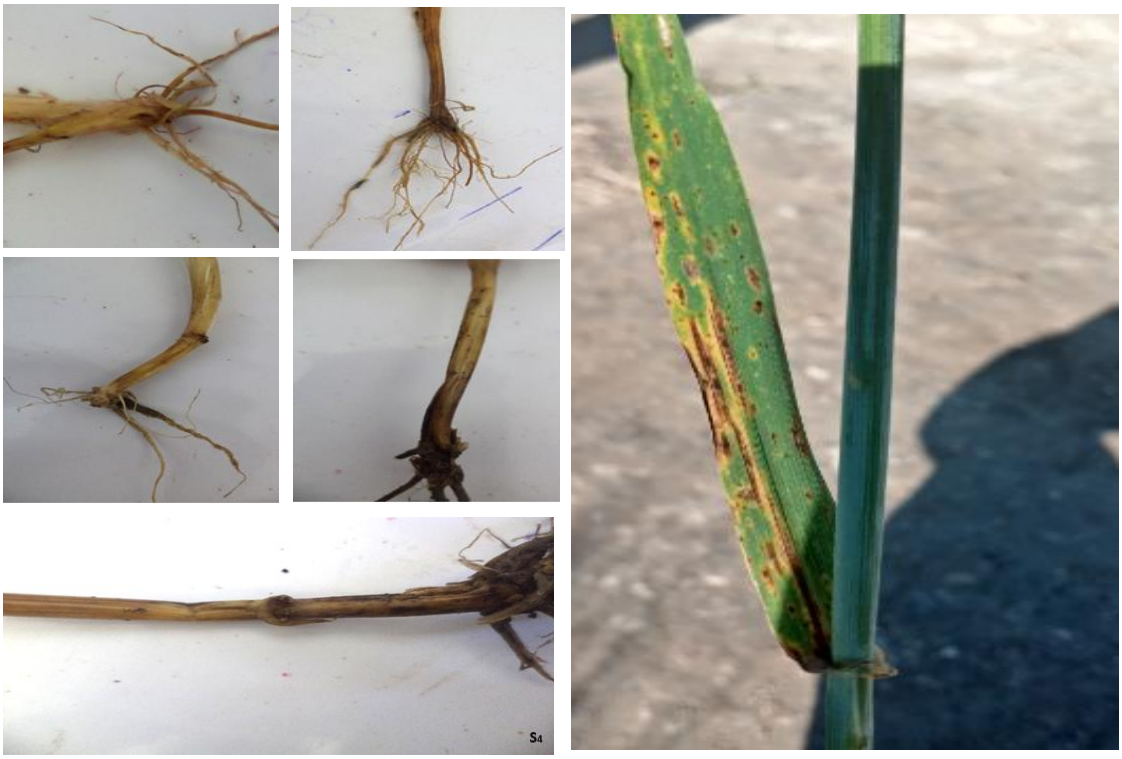

Fig. 1. Different types of symptoms induced by *C. spicifera* in durum wheat: root necrosis and foliar lesions

Disease indices observed in wheat and barley plants from seed inoculated with *C. spicifera* isolates ranged from 43.3 to 75%. The index is minimal in barley inoculated with H5C BD Megren isolate, originating from durum wheat and maximum in common wheat inoculated with H10C Barley Ouazzane isolate. In general, disease indices are high in durum wheat (range 61.6-70) and common wheat (vary between 60-75%) and decrease in barley (vary between 43.3-61.6%).

Isolates of *C. spicifera* were also able to alter the leaf area of the tested varieties of wheat (durum and soft) and barley. Many blackish brown spots with yellowish outline are observed on the leaves. They are of variable shapes, initially elliptical and tapered, then they elongate and fuse together forming important necrotic lesions. Severely infected leaves eventually dry out.

The number of foliar lesions induced by the different isolates of *C. spicifera* is very high in the wheat and barley plants from the inoculated seeds. These lesions are sporulating, the number of spores formed vary between 6.37 and 6.11 10^6 spores / cm^2.

These results indicate that isolates of *C. spicifera* are able to induce typical root rot symptoms in wheat and barley, some noted symptoms at the roots of the neck and under the collar of the plants. Severity class S4 is characterized by the development of rot at the base of plants, chlorosis and stunting in the aerial parts, was observed in the wheat and barley plants inoculated with these isolates. The incidences and indices of the disease can reach 100% and 75% respectively.

Fig. 2. Different types of symptoms induced by *C. spicifera* in barley: root necrosis and foliar lesions

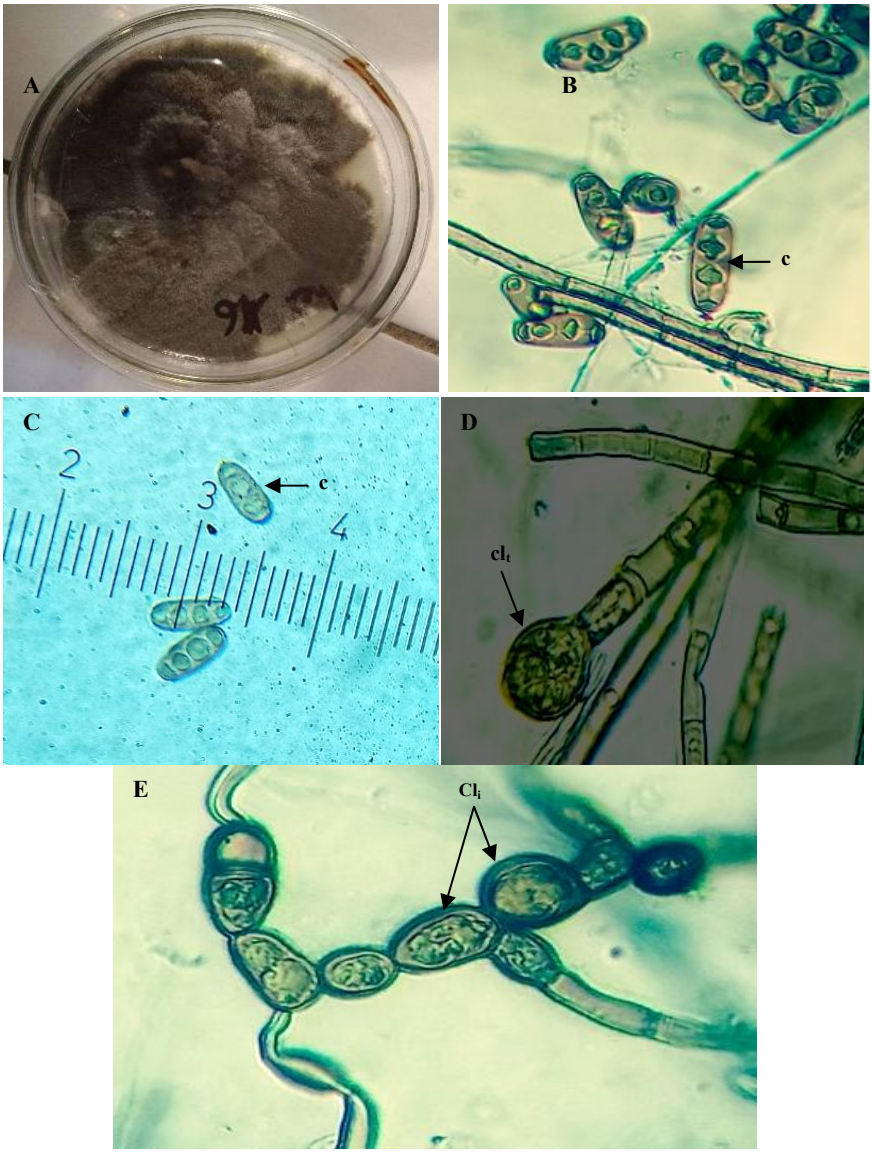

Fig. 3. Different life cycle components of *C. spicifera*: colony on PSA (A); conidia (B and C); chlamydospores (D clt: terminal chlamydospores and E cli: intercalated chlamydospores)

DISCUSSION AND CONCLUSION

In the north-western region of Morocco, the fungal complex responsible for root rot of wheat and barley has hosted *C. spicifera.* The percentages of isolation of this pathogen from wheat (hard and soft) and barley roots are almost identical to those noted for fungal species in the complex, *Bipolaris sorokiniana* and *Fusarian* species [27]. The isolates of *C. spicifera* collected form on the PSA medium intercalated or isolated chlamydospores at the end of the mycelial filaments. These chlamydospores have never been, to our knowledge, cited in the literature about C. *spicifera.* Thus, it seems important to study the process of formation of these chlamydospores on different culture media, to follow the different stages of their differentiations and to give a detailed microscopic description of their microscopic characters. Indeed, this process of

clamydogenesis could give an idea on the number of formed conservation cells, resistance cells that in nature will ensure the conservation of the pathogen from one year to another in the soil or on the debris of the previous culture. It is also worth mentioning that in *Bipolaris sorokiniana* (synonym *Helminthosporium sativum*), chlamydospores form from conidia and ensure the survival of this pathogen in the soil [32].

*C. spicifera*is therefore present in the northwestern region of Morocco. The study of the pathogenicity of certain isolates of this pathogen taking into account the induced severity, the incidence and index of the disease showed that each isolate resulted in different responses in the plants of the studied host species (wheat and barley). The S4 class of severity has been observed, with varying percentages, in almost all wheat (hard and soft) and barley plants from inoculated grains. The incidences and indices of the disease are also high. The isolates of *C. spicifera* studied induced typical symptoms of root rot and showed an ability to produce conidia on the leaves of the host. The pathogen migrates over time from roots to higher plant levels and causes blackish brown foliar lesions that fuse with time and become elongated. All these foliar lesions are sporulating. Waggoner and Horsfall [33] and Kranz et al. [34] noted that the numerical values of sporulation are essential for the simulation of epidemiological programs. The most infectious species in the case of an epidemic are those that attack a larger part of the host tissue and allow multiplication of the inoculum on foliar lesions [35].

Bipolaris sorokiniana is the pathogen of the fungal complex responsible for the most studied root rot, it induces necrotic lesions on the roots and foliar lesions on wheat (durum and soft) and barley plants. The description of the symptoms developed on the roots and foliage of wheat and barley plants following artificial inoculation with *C. spicifera* has not been well studied in the literature. In contrast, foliar symptoms induced by this pathogen have been reported in other host plant species. In *Citrullus lanatus*, for example, foliar symptoms induced by *C. spicifera* are brown to black, irregularly shaped lesions [20]. In banana, *C. spicifera* is responsible for lenticular spots on the limbus and petiole, which have a light brown to whitish center, have a distinct dark border, and are often surrounded by a diffuse yellowish halo [24]. According to these authors, pathogenic strains of *C. spicifera* on banana can have two origins, either they already existed among the parasitic mycoflora of the leaf mass and are selected by the cultivated variety, or non-pathogenic lines have been progressively adapted to the introduced variety.

Ennaffah et al. [6] and Ouazzani Touhami et al. [18] described symptoms in different varieties of rice inoculated with isolates of *C. spicifera* collected from foliar lesions of rice. In general, these symptoms occur in the form of small brownish spots, of tapered shape, which merge to give necrotic patches that come together over time and lead to drying of leaves. These isolates of *C. spicifera* were able to sporulate foliar lesions to varying degrees depending on their virulence and the sensitivity and resistance of different rice varieties [18]. Gnancadja-André et al. [26] noted that *C. spicifera* and other fungal species (*Helminthosporium oryzae, H. sativum, H. australiensis, Curvularia lunata, Fusarium moniliforme* and *Epicoccum nigrum*) isolated from foliar lesions and rice seed are responsible for the tarnishing of rice grains (*Oryza sativa* L.) According to these authors, *C. spicifera* was able to cause tarnishing in 21% of the inoculated grains and the tarnish index calculated in rice grains, Taibonnet variety, inoculated with *C. spicifera* is of the order of 21%. In Iraq, Hamdia Z. Ali et al. [36] considered *C. spicifera* as a foliar pathogen of rice.

Berber et al. [37] reported that *C. spicifera* is capable of producing tinged, brown spots on sorghum that may over time join to form necrotic spots with dark brown outline and whitish center. In 2009, *C. spicifera* was reported for the first time in Turkey, Sakary Province, as a causal agent for foliar lesions in sorghum [31]. The incidence and severity of the disease induced by this pathogen in sorghum were evaluated at 45 and 25-75% respectively [31]. *C. spicifera* is also able to affect *Capsicum annum* [38] and *Lens culinaris* [39]. The pathogen was isolated from *Ficus retusa nitida* and was able to induce on the leaves of this plant species brown lesions, longitudinal, develop at the beginning of the periphery and

progress with time towards the center of the leaves. In the United States of America, Vu et al. [40] reported for the first time *C. spicifera* as the pathogen responsible for foliar lesions of *Panicum virgatum* (Switch grass).

The host range of *C. spicifera* is very diverse. *C. spicifera* was isolated from 77 host plants, air and soil [30] and was isolated from *Hibiscus rosa-sinensis* [19]. In Korea, *C. spicifera* has been isolated among other fungal species from the grains of some grasses, and its pathogenicity has been confirmed with respect to rice, maize, Bermuda grass, sorghum and tall fescue [12]. But according to these authors, *C. spicifera* could not infect and induce leaf symptoms in wheat and blue grass. In Burkina Faso, *C. spicifera* has been isolated from seeds and collets of *Jatropha curcas* L. plants. [41]. In Nebraska, Amaradasa and Amundsen [42] reported *C. spicifera* as a fungal agent responsible for burning leaves of Bouteloua typists growing in lawns. According to these authors, at the beginning of the infection, the leaves show dark brown oblong spots that meet and then dry out and the tillers decay. In Egypt, Ismail et al. [43] reported that *C. spicifera* is responsible for foliar lesions of tomatoes. These lesions are brown to black with a yellowish halo. In Iran, Ayoubi et al. [44] considered *C. spicifera* and *C. inaequalis* as fungal pathogens responsible for leaf blight and strawberry fruit rot.

Inoculation tests show that isolates of *C. spicifera* have significant pathogenicity to wheat and barley. These isolates were shown to be able to initially attack wheat and barley roots and to induce sporulating leaf lesions; they are therefore able to give a secondary inoculum which can initiate the disease on other healthy leaves of wheat or barley. With time and in the absence of an effective control program, leaf blight, caused by *C. spicifera,* will probably become important and take up more of the other known diseases of cereals. In this sense, it is important to consider this pathogen in programs for the development of new resistant varieties of wheat and barley and to monitor the possible occurrence of other virulent pathogens. Generalization of certain susceptible varieties of wheat and barley would likely result in an increase in the population of *C. spicifera*, which may be a threat to wheat and barley growing over time. The danger of such a species does not seem immediate, but its presence at ground level and at all parts of the plants should be carefully monitored.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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