

## Pathogenicity of four Fungal Species on Fruits and Leaves of the Olive Tree (*Olea Europaea* L.)

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### ABSTRACT

*Pathogenicity of Alternaria alternata, Aspergillus flavus, Cladosporium sp. and Colletotrichum gloeosporioides isolated from olive fruits was studied by inoculating olive fruits and leaves. Three techniques were used to inoculate the fruits (mycelial plug on the intact and the injured olives and by injecting them with the spore's suspension) and two techniques for the leaves (mycelial plug on the intact and the injured olive leaves). Alternaria alternata had no pathogenic effect neither on leaves nor on the olives. Also, Aspergillus flavus was not able to colonize or to provoke any symptoms on the intact fruits, this fungus provoke green mold when the epicarp was artificially injured with a colonization percentage of 40 % and forms the conidiophores producing conidia on the olive fruit, the depth of this mold was 0.4 cm affecting all the fruit tissues. Cladosporium sp. has shown a brown rot on the olive fruit and the colonization percentages of this fungus on the olives were 65, 70 and 60% using respectively the techniques 1, 2 and 3 of inoculation. Colletotrichum gloeosporioides was able to affect the olives and to show a rot on them. Ten days after inoculation of the intact and the injured leaves, the estimated disease severity caused by Colletotrichum gloeosporioides on leaves of Dahbia and Haouzia olive tree was 100 %. The possibility of decreasing the olive oil quality because of these fungal species was discussed in this study.*

**Key words:** Olive tree, *Aspergillus flavus*, *Alternaria alternata*, *Colletotrichum gloeosporioides*.

### INTRODUCTION

Olive tree (*Olea europaea* L.), is an important oil-producing crop in Mediterranean countries such as Spain, Italy, Greece, Turkey, and many other countries such as USA (California), Argentina, Chile, Australia and New Zealand<sup>52,32</sup>. The Moroccan olive industry is largely traditional supplying the needs of the population where olives and olive oil form an integral part of the diet. National production is estimated at 60,000 tonnes of oil<sup>13</sup>. Olive production occurs throughout the country with a very large number of smaller producers. Olives are everywhere with small to medium sized groves on the coastal plains, on mountain slopes, on roadsides and as part of town streetscapes and even in the Saharan oases<sup>13</sup>.

A lower prevalence of non-communicable diseases such as cardiovascular disease and certain types of cancers have been demonstrated in countries residing in the Mediterranean region in comparison to other parts of the world<sup>11,28</sup>. This lowered incidence has been partially attributed to the regular intake of virgin olive oil as part of a traditional Mediterranean diet<sup>49,28,45,14</sup>.

Several biotic and abiotic factors affect olive tree consequently reducing the tree health and vigour, crop yield and quality of the extracted oil<sup>23,20,36,34,38</sup>. Olive cultivar is one of the most important factors that affect the quality of olive oil<sup>34</sup>. In addition to the effects of cultivar, the degree of fruit ripeness, and the industrial processes used for oil extraction, as well as the quality of the fruit from which oil is extracted has a great effect on olive oil quality<sup>16</sup>. Diseases of olive drupes can cause financial losses through direct loss of rotted drupes, reduced cosmetic value of table olives and reduced quality of the oil due to fungal infections<sup>25</sup>.

Diverse fungal groups have been reported to cause fruit rot on olive, including species of Botryosphaeriaceae such as *Botryosphaeria*, *Diplodia*, *Lasiodiplodia*, *Macrophomina*, *Neofusicoccum* and *Camarosporium*; Glomerellaceae including *Colletotrichum*; Mycosphaerellaceae incl. *Pseudocercospora* and several other fungal species with minor importance such as *Fusicladium oleagineum*, *Alternaria* spp., *Aureobasidium pullulans*, *Epicoccum nigrum*, *Cladosporium herbarum* s.l., *Capnodium elaeophilum*, *Truncatella angustata*, *Pilidium concavum* and *Pestalotia fici*<sup>2,3,4,5,8,9,29,30,39</sup>.

The aim of this work was to study the pathogenicity of four fungal species on the fruits and leaves of the olive tree.

## MATERIALS AND METHODS

### Pathogen isolation

The isolation of fungal species from fruits showing rot symptoms in Gharb (Fig. 1A) and Zoumi (Suburb of Ouazzane) (Fig. 2B). Fragment of the olives showing rots were washed with water, disinfected with alcohol for five minutes, put on sterile distilled water and then dried with sterile filter paper. Then, they were put on PSA agar plates (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g Agar-agar, and 1000 mL distilled water) and incubated on darkness at 28 degree. The developing colonies were then observed for the species determination.

### Pathogenicity test on the olive fruits

Using two inoculation techniques on the olive fruits, pathogenicity test was studied:

**Technique 1:** The fruits were washed in running water and surface disinfected for 1 min in 75% Alcohol. Then washed with sterile distilled water and dried with sterile filter paper. Twenty olive fruits were inoculated with a mycelial plug (5 mm in diameter) of each fungal species culture that was placed over the intact olive fruit. Twenty other non-inoculated fruits (only plugs of PSA culture), served as a control. The fruits were placed in humid chamber at the room temperature.

**Technique 2:** Same inoculation processes of the technique 1 with making an artificial injury on the olive fruits (2 mm injury diameter).

**Technique 3:** Conidia suspension with a concentration of  $10^6$  conidia/ mL was prepared; inoculation test was performed on twenty surface-sterilized healthy olive fruits. Fresh olive fruits were dipped in 70% alcohol for 20–30 sec and rinsed three times in sterilized water. 0.1 mL of the spore suspension was injected inside the fruits and 0.1 mL of distilled water was injected inside the fruits used as a control. After the inoculation test, fruits were placed in sterilized Petri dishes containing sterilized filter paper (Watman N° 2). The filter paper was kept wet during the experiment and the Petri dishes were incubated on the lab bench under daylight regime in the laboratory conditions (20 to 24 degrees).

Eight days after the inoculation of the olives, the colonization percentage of the used fungal species on the olive fruits and the rot depth were estimated in every treatment relative to the control.

### Pathogenicity test on the olive leaves

Olive leaves for each of Dahbia and Haouzia varieties were washed in tap water and surface disinfected for 1 min in 75% Alcohol. Then washed with sterile distilled water and dried with sterile filter paper. Thirty olive leaves were inoculated with a mycelial plug (5 mm in diameter) of each fungal species. Mycelial plug placed in the middle of intact (Technique 4) and on the injured olive leaves (Technique 5).

Thirty other non-inoculated leaves (only plugs of PSA culture), served as control. The leaves were placed in humid chamber at room temperature (24 °C).

### Measured parameters

The disease severity was scored after 10 days of inoculation using the scale of Stover modified by Gauhl et al.<sup>18</sup> :

$$\text{Infection index} = \sum nb \times 100 / (N-1) T$$

n: number of leaves in each grade.

b: grade.

N: number of grades used in the scale (6).

T: total number of leaves scored.

Leaves those had shown lesions were cut into pieces of 1 cm<sup>2</sup> and placed in 9 cm 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 × 400 with 10 counting of each sample.

## RESULTS

Data in Table 1 shows that the most dominate species related to the olives rot in Gharb was *Aspergillus flavus* (65%) and *Colletotrichum gloeosporioides* had the highest isolation percentage (64%) from the fruits rot of the olive tree cultivated in Zoumi.

*Alternaria alternata*, *Aspergillus flavus*, *Cladosporium* sp. and *Colletotrichum gloeosporioides* were used to inoculate the olives.

Basing on the colonization percentage of the fungal species isolated from olive fruits, *Alternaria alternata* was not able to colonize the olive fruits. Also, *Aspergillus flavus* was not able to colonize or to provoke any symptoms on the intact olive fruits, this fungus provoke green mold when the epicarp was artificially injured with a colonization percentage of 40 % and forms the conidiophores producing conidia on the olive fruit (Figure 2A, Table 2). The depth of this mold was 0.4 cm (Table 3) affecting all the fruit tissues (Figure 2B). Also, using the technique 3 of inoculation, *A. flavus* was able to colonize 50 % of the olives (Table 2) and to affect all the fruit tissues with a rot depth of 0.3 cm (Table 3).

*Cladosporium* sp. has shown a brown rot on the olives (Figure 2C) no matter the inoculation technique was. Colonization percentages of this fungus were 65, 70 and 60% on the olives inoculated using respectively the techniques 1, 2 and 3 (Table 2). This rot affect all the olive fruit (Figure 2D) with a depth of 0.4, 0.35 and 0.35 on the olives inoculated using respectively the techniques 1, 2 and 3 (Table 3). Fruit becomes mummified in the finish of the fungal cycle with a reduction of the fruit caliber.

Same as *Cladosporium* sp., *Colletotrichum gloeosporioides* was able to affect the olives and to show a rot on them (Figure 2F) no matter the inoculation technique was. This fungus colonized 50, 60 and 70% of the olives surface after eight days of inoculation using respectively the techniques 1, 2 and 3 with a rot depth of 0.4 cm (Table 3). After twelve days of inoculation, *C. gloeosporioides* produced orange acervuli on the olive fruits (Figure 2F).

Non inoculated olive leaves had shown no symptoms. *C. gloeosporioides* have affected both of Haouzia (Figure 3B) and Dahbia (Figure 3D) no matter the technique of inoculation was. Ten days after inoculation of the intact and the injured olive leaves, the estimated disease severity on leaves of Dahbia and Haouzia olive tree was 100 % (Table 4),

Ten days after inoculation, leaf spots are pale brown to brown, in the surface of the inoculated leaves (Figure 3) surrounded by dark brown zone. Minute orange dots (acervuli) scattered on the leaf spot, *C. gloeosporioides* produced conidia abundantly on the surface of the olive leaves. The concentration of conidia formed on the leaf surface was  $8 \cdot 10^7$  and  $1.24 \cdot 10^8$  conidia.cm<sup>-2</sup> respectively for the intact Dahbia and Haouzia olive leaves. The experiment had shown no difference between the conidia's concentration on the intact and the injured olive leaves. Thus, conidia concentration of the *C. gloeosporioides* was respectively  $1.73 \cdot 10^8$  and  $1.47 \cdot 10^8$  conidia.cm<sup>-2</sup> on the injured Dahbia and Haouzia olive leaves.

Fig. 1: Olives rot in Gharb (A) and Zoumi region (B)

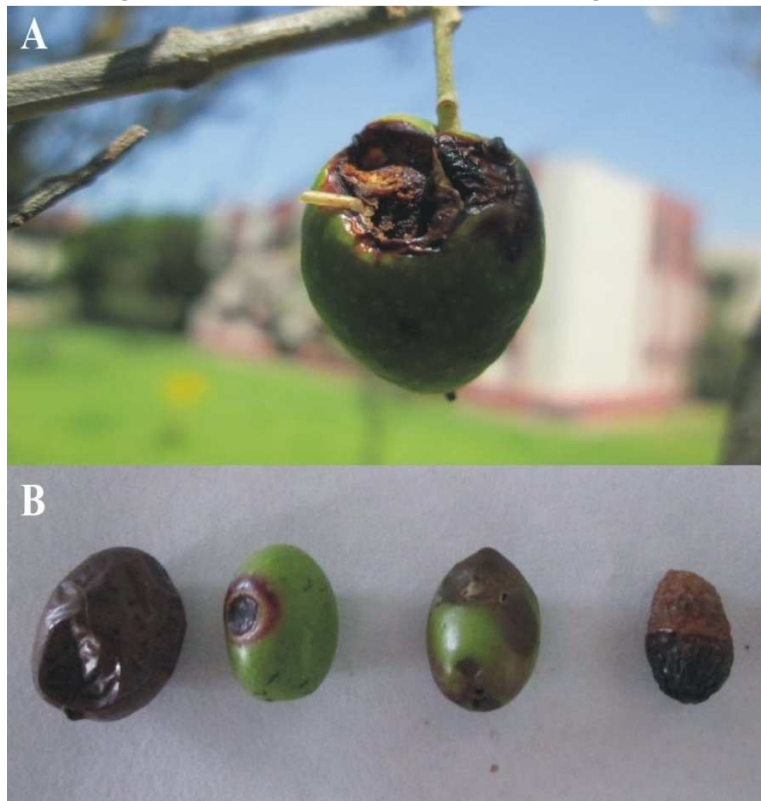


Fig.2: Olive fruits after eight days of inoculation. Green mold on (A) and the inside the olive fruit (B) caused by *Aspergillus flavus*; brown rot on (C) and the inside the olive fruit (D) caused by *Cladosporium* sp.; anthracnose symptom on the olive fruit (E); *Colletotrichum gloeosporioides* acervuli formed on the olive fruit after 12 days of inoculation (F).



**Table 1. Isolation percentage of different fungal species isolated from fruits of the olive tree cultivated in Gharb and in Zoumi regions**

Fungal species	Kenitra (%)	Zoumi (%)
<i>Alternaria alternata</i>	12 <sup>b</sup>	2 <sup>c</sup>
<i>Colletotrichum gloeosporioides</i>	-	64 <sup>a</sup>
<i>Cladosporium</i> sp.	-	25 <sup>b</sup>
<i>Penicillium</i> sp.	3 <sup>b</sup>	-
<i>Aspergillus flavus</i>	65 <sup>a</sup>	-
<i>Circinella</i> sp.	5 <sup>b</sup>	-

The results of the same column followed by different letters differ significantly at 5%.

Tech.: Technique; C.P.: Colonization percentage.

**Table 2. Measured parameters after eight days of the olive fruit inoculation with four fungal species**

		Inoculated olive fruit ( <i>Alternaria alternata</i> )	None inoculated olive fruit ( <i>Alternaria alternata</i> )	Inoculated olive fruit ( <i>Aspergillus flavus</i> )	None inoculated olive fruit ( <i>Aspergillus flavus</i> )	Inoculated olive fruit ( <i>Cladosporium</i> sp.)	None inoculated olive fruit ( <i>Cladosporium</i> sp.)	Inoculated olive fruit ( <i>Colletotrichum gloeosporioides</i> )	None Inoculated olive fruit ( <i>Colletotrichum gloeosporioides</i> )
Tech. 1	C.P. (%)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	65 <sup>a</sup>	0 <sup>a</sup>	50 <sup>a</sup>	0 <sup>a</sup>
Tech. 2	C.P. (%)	0 <sup>a</sup>	0 <sup>a</sup>	40 <sup>b</sup>	0 <sup>a</sup>	70 <sup>a</sup>	0 <sup>a</sup>	60 <sup>a</sup>	0 <sup>a</sup>
Tech. 3	C.P. (%)	0 <sup>a</sup>	0 <sup>a</sup>	50 <sup>b</sup>	0 <sup>a</sup>	60 <sup>a</sup>	0 <sup>a</sup>	70 <sup>a</sup>	0 <sup>a</sup>

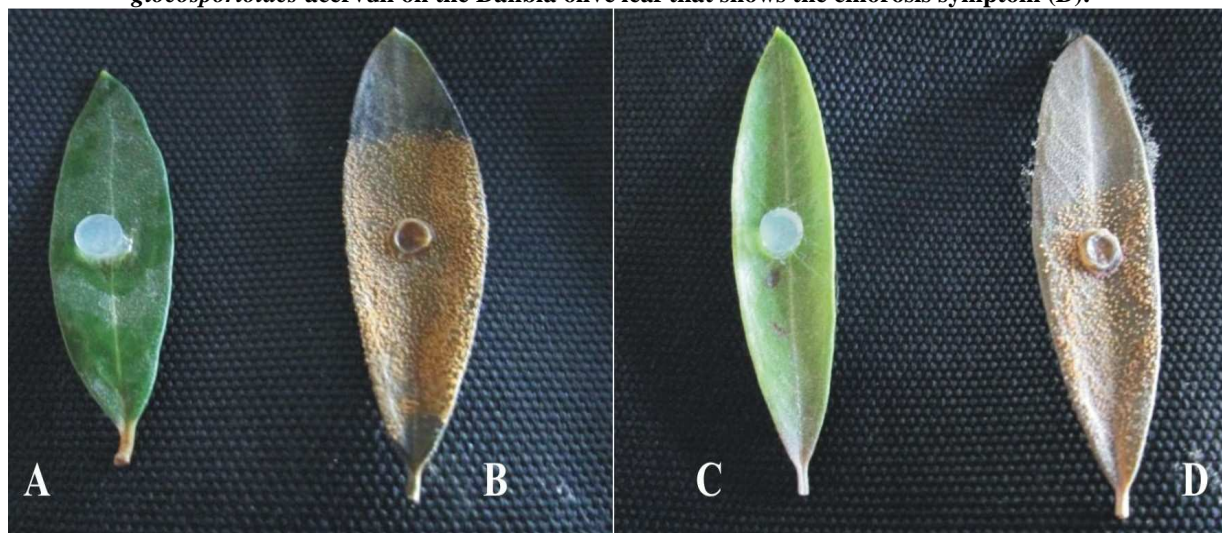
The results of the same column followed by different letters differ significantly at 5%.

**Table 3. Depth of the olive rot created by the fungal species after eight days of inoculation**

		Inoculated olive fruit ( <i>Alternaria alternata</i> )	None inoculated olive fruit ( <i>Alternaria alternata</i> )	Inoculated olive fruit ( <i>Aspergillus flavus</i> )	None inoculated olive fruit ( <i>Aspergillus flavus</i> )	Inoculated olive fruit ( <i>Cladosporium</i> sp.)	None inoculated olive fruit ( <i>Cladosporium</i> sp.)	Inoculated olive fruit ( <i>Colletotrichum gloeosporioides</i> )	None Inoculated olive fruit ( <i>Colletotrichum gloeosporioides</i> )
Tech.1	Depth (cm)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0.4 <sup>a</sup>	0 <sup>a</sup>	0.4 <sup>a</sup>	0 <sup>a</sup>
Tech.2	Depth (cm)	0 <sup>a</sup>	0 <sup>a</sup>	0.4 <sup>b</sup>	0 <sup>a</sup>	0.35 <sup>a</sup>	0 <sup>a</sup>	0.4 <sup>a</sup>	0 <sup>a</sup>
Tech.3	Depth (cm)	0 <sup>a</sup>	0 <sup>a</sup>	0.3 <sup>b</sup>	0 <sup>a</sup>	0.35 <sup>a</sup>	0 <sup>a</sup>	0.4 <sup>a</sup>	0 <sup>a</sup>

The results of the same column followed by different letters differ significantly at 5%.

**Fig.3: Olive leaf of the Haouzia control (A); formation of the *C. gloeosporioides* acervuli on the Haouzia olive leaf that shows the chlorosis symptom (B); olive leaf of the Dahbia control (C); formation of the *C. gloeosporioides* acervuli on the Dahbia olive leaf that shows the chlorosis symptom (D).**





**Table 4. Severity diseases and conidia concentration on the leaf surface of the inoculated and none inoculated Dahbia and Haouzia olive leaves with *Colletotrichum gloeosporioides***

		Inoculated olive leaves (Dahbia)	Non inoculated olive leaves (Dahbia)	Inoculated olive leaves (Haouzia)	Non inoculated olive leaves (Haouzia)
Technique 4	Severity index (%)	100 <sup>a</sup>	0 <sup>b</sup>	100 <sup>a</sup>	0 <sup>b</sup>
	Conidia concentration on the leaf surface (conidia.cm <sup>-2</sup> )	8. 10 <sup>7</sup> <sup>a</sup>	0 <sup>b</sup>	1.24 10 <sup>8</sup> <sup>a</sup>	0 <sup>b</sup>
Technique 5	Severity index (%)	100 <sup>a</sup>	0 <sup>b</sup>	100 <sup>a</sup>	0 <sup>b</sup>
	Conidia concentration on the leaf surface (conidia.cm <sup>-2</sup> )	1.73. 10 <sup>8</sup> <sup>a</sup>	0 <sup>b</sup>	1.47 10 <sup>8</sup> <sup>a</sup>	0 <sup>b</sup>

The results of the same line followed by different letters differ significantly at 5%.

## DISCUSSION AND CONCLUSION

Olive fruits are very susceptible to fungal infections during the ripening period. Plant pathogenic fungi secrete a wide arrays of extracellular enzymes to degrade cellular tissue and absorb nutrients<sup>1</sup>. Fungal species produces a range of hydrolytic, proteolytic and cellulolytic enzymes and metabolites to facilitate penetration and colonization of host tissues<sup>1</sup>, which may result in reduced quality of the extracted oil.

The used strain of *Alternaria alternata* had no effect on the fruits and on the leaves of the olive tree. In the other hand, Moral *et al.*<sup>29</sup> demonstrate the high susceptibility of Olive cultivar FS-17 to *Alternaria alternata* in southern Spain. *Alternaria alternata* may secrete some Mycotoxins in infected olive fruits and they are possible to be transferred into oil<sup>7</sup>. *Alternaria* species produce different mycotoxins such as Alternariol (AOH), Alternariol methyl ether (AME), Altenuen (ALT), and Tenuazonic acid (TeA) which leave harmful effects on bacterial, human and animal cell cultures<sup>24,50</sup>.

*Cladosporium* sp. was responsible to the olive rot in Iran<sup>38</sup> and in Australia (Sergeeva *et al.*, 2008b). The obtained results showed that *Cladosporium* sp. was able to infect both of the intact and the injured olives.

*Alternaria* and *Cladosporium* have been associated with the end or nose rots seen on several cultivars the fungi recovered from these including<sup>38</sup>. Also, Torbati *et al.*<sup>46</sup> reported that both of *Alternaria alternata* and *Cladosporium cladosporioides* provoke rot on the olive fruits. These fungi can be both primary and secondary invaders, and further work needs to be undertaken to confirm their importance as pathogens<sup>38</sup>.

*Aspergillus flavus* has shown a green mold on the olives, it is also responsible of peaches and plum rots in different countries<sup>22,28,42</sup>. This pathogen is one of the major fungi species producing aflatoxin, a group of toxic, carcinogenic compounds<sup>12,34,48</sup>. The obtained results showed that *A. flavus* couldn't degrade the olive fruit epicarp, the contamination of olives with *A. flavus* necessitates injuries. This was also reported by Lazzizera *et al.*,<sup>25</sup> those announced that most of fungal species causing fruit rot of olive are common saprophytes or secondary invaders normally penetrating through injuries made by biotic or abiotic factors. Anthracnose caused by *Colletotrichum acutatum* and *C. gloeosporioides* is a common and widespread fruit rot disease of olives (*Olea europaea* L.) in most olive growing regions in the world<sup>41</sup>. The most typical symptoms of olive anthracnose (OA) are fruit rot and mummification. In moist conditions, infected fruits show a soft to dark brown rot with an abundant production of an orange gelatinous matrix embedding conidia emerging from acervuli, while, in dry conditions, the fruits mummify and lose weight due to dehydration. Affected fruits fall prematurely to the ground and only a few mummies remain attached to the tree. Fruit drop may also be the consequence of infections in the peduncle<sup>32</sup>.

Anthracnose has been previously recorded on olive leaves in Mediterranean countries such as Portugal, Spain and Italy<sup>40,44,45</sup>. Olive anthracnose caused by *Colletotrichum* spp. is becoming an important disease of fruits causing major losses in yield of olives and the quality of olive oil<sup>21,44</sup>.

*C. gloeosporioides* was reported for the first time as the responsible agent of the olives anthracnose in Morocco by Achbani *et al.* (2013) in the region of Ouazzane, the obtained results showed that both of *Colletotrichum gloeosporioides* and *Cladosporium* sp. were isolated from the same rot symptoms and both of them provoked rots on olives without any injuries.

*C. gloeosporioides* was able to affect the leaves showing Chlorosis symptoms and it was also able to form acervuli on the olive leaves, it was also reported by Sergeeva *et al.*<sup>40</sup> in Australia leaves and flowers. Infected leaves were considered as inoculum sources for fruit anthracnose (Cacciola *et al.* 1996). Moreover, a very poor olive oil quality is obtained from olives harvested in anthracnose affected areas because of alterations in oil color (red), acidity, and organoleptic characteristics<sup>10,17</sup>. These infected leaves can be also an inoculum source to affect the olive flowers and fruits in the flowering and fructification seasons. *Colletotrichum gloeosporioides* has a latent period and spores can survive saprophytically for long periods<sup>36</sup> and heavy infections of anthracnose cause massive defoliation. Infections begin by the spores germinating from the acervuli on the fruits, leaves and young shoots<sup>26</sup>.

Some of the tested species provoked rots on the olive fruits with and without injuries; several biotic and abiotic factors may cause injury on olive fruits such as pest insects, hail, and many others<sup>46</sup>. Olive fruits are very susceptible to fungal infections during the ripening period. Plant pathogenic fungi secrete a wide arrays of extracellular enzymes to degrade cellular tissue and absorb nutrients<sup>1</sup>. Fungal species produces a range of hydrolytic, proteolytic and cellulolytic enzymes and metabolites to facilitate penetration and colonization of host tissues<sup>1</sup>, which may result in reduced quality of the extracted oil.

#### Acknowledgments

This study was conducted under the project 'Rhizolive: Selection and use of soil rhizospheric microorganisms to optimize the arbuscular mycorrhization of the olive tree in Morocco's soils funded by Hassan II Academy of sciences and technology.

#### REFERENCES

1. Agrios, G.N. Plant pathology. New York (NY): Academic Press/Elsevier (2005)
2. Arzanlou, M. Torbati, M. Jafary, H. Fruit rot of olive (*Olea europaea* L.) caused by *Truncatella angustata*. *Plant Pathol Quar*, **2**: 117–123 (2012)
3. Arzanlou, M. Torbati, M. Jafary, H. Fruit rot on olive caused by *Pilidium concavum* in Iran. *Australasian Plant Dis*. **8**: 117–121 (2013)
4. Athar, M. Infestation of olive fruit fly, *Bactrocera oleae*, in California and taxonomy of its host trees. *Agric Conspec Sci*. **70**:135–138 (2005)
5. Avila, A. Groenewald, J.Z. Trapero, A. Crous, P.W. Characterization and epitypification of *Pseudocercospora cladosporioides*, the causal organism of Cercospora leaf spot of olives. *Mycol Res* **109**: 881–888 (2005)
6. Benkirane, R. Contribution à l'étude des maladies du riz au Maroc. Cas de la pyriculariose due à *Pyricularia oryzae*. Thèse de 3ème cycle. Université Ibn Tofail, Faculté des Sciences, Kénitra, Maroc, 145 (1995)
7. Bottalico, A. and Logrieco, A. Mycotoxins in *Alternaria alternata* - infected olive fruits and their possible transfer into oil. **23(3)**: 473–479 (1993)
8. Chattaoui, M. Rhouma, A. Krid, S. Ali Triki, M. First report of fruit rot of olive caused by *Botryosphaeria dothidea* in Tunisia. *Plant Dis* **95**: 770–770(2011)
9. Chliyeh, M. Rhimini, Y. Selmaoui, K. Ouazzani Touhami, A. Filali-Maltouf, A. El Modafar, C. Moukhli, A. Oukabli, A. Benkirane, R. and Douira, A. First report of *Pestalotia fici* causing leaf chlorosis and fruit rot on olive (*Olea europaea* L.) in Morocco. *International Journal of Recent Scientific Research*. **5(1)**: 136-141 (2014)
10. De Andrés, F. Enfermedades, Y. plagas del Olivo. Riquelme Y. Vargas Ediciones S.L. Jaén, Spain (1991)
11. De Lorgeril, M. Salen, P. Martin, J.L. Monjaud, I. Delaye, J. Mamelle, N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation*, **99**: 779–785 (1999)
12. Diener, U.L. Cole, R.J. Sanders, T.H. Payne, G.A. Lee, L.S. Klich, M.A. Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Ann. Rev. Phytopathol*. **25**: 249-270 (1987)
13. Fennell, J. Olive production Morocco and Spain. Principal Horticulturist Agriculture, Food & Wine Primary Industries & Resources SA. **36**: (2007)

14. Fung, T.T. Rexrode, K.M. Mantzoros, C.S. Manson, J.E. Willett, W.C. Hu, F.B. Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. *Circulation* **119**: 1093–1100 (2009)
15. Galeone, C. Talamini, R. Levi, F. Pelucchi, C. Negri, E. Giacosa, A. Montella, M. Franceschi, S. La Vecchia, C. Fried foods, olive oil and colorectal cancer. *Ann. Oncol.* **18**: 36–39 (2006)
16. Garcia, J.M. Yousfi, K. The postharvest of mill olives. *Grasas Aceites.* **57**:16–24 (2006)
17. García-Figueres, F. Micosis de las aceitunas y su incidencia en la calidad del aceite. *Phytoma España,* **102**:171-175 (1998)
18. Gauhl, F. Pasberg-Gauhl, C. Vuylsteke, D. and Ortiz, R. Multilocational evaluation of black Sigatoka resistance in banana and plantain. IITA Research Guide 47. 2nd edition. Training Program, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 59 (1995)
19. Graniti A, Frisullo S, Pennisi A.M. Magnano di San Lio G Infection of *Glomerella cingulata* on olive in Italy. *OEPP/EPPO Bulletin,* **23**: 457–465 (1993)
20. Gutierrez F, Aranaud T, Albi AM. Influence of ecologic cultivation on virgin olive oil quality. *J Am Oil Chem Soc.* **76**: 617–621 (1999)
21. Iannota, N. Perri, E. Seriani, R. Tocci, C. Influence of *Colletotrichum gloeosporioides* (Penzig) and *Camarosporium dalmatica* (Thum) attacks on olive quality. *Acta Horticulturae,* **474**: 573–576 (1999)
22. Jammaluddin, A. Organic acid composition of peach fruits during infection by *Aspergillus flavus*. *Indian Phytopathology* **32**: 490 (1980)
23. Kiritsakis, A. Olive oil: from the tree to the table, 2nd ed. Trumbull (CT): Food and Nutrition Press (1998)
24. Kralova, J. Hajslova, J. Poustka, J. Hochman, M. Bjelkova, M. Odstrcilova, L. Occurrence of *Alternaria* toxins in fibre flax, linseed, and peas grown in organic and conventional farms: monitoring pilot study. *Czech J Food Sci.* **24**: 288–296 (2006)
25. Lazzizera, C. Frisullo, S. Alves, A. Phillips, A.J.L. Morphology, phylogeny and pathogenicity of *Botryosphaeria* and *Neofusicoccum* species associated with drupe rot of olives in southern Italy. *Plant Pathol* **57**: 948–956 (2008)
26. Martelli, G.P. A first contribution to the knowledge of the biology of *Gloeosporioides olivarum*. *Phytopathologia Mediterranea* **1**: 101-112 (1960)
27. Matalas, A.-L. Zampelas, A. Stavrinou, V. Wolinsky, I. The Mediterranean Diet: Constituents and Health Promotion; CRC Press: Boca Raton, FL, USA, 2001.
28. Michailides, T. Thomidis, T. First report of *Aspergillus flavus* causing fruit rots of peaches in Greece. *New disease reports.* **14**: 13 (2006)
29. Moral, J. De La Rosa, R. Leon, L. Barranco, D. Michailides, T.J. Trapero, A. High susceptibility of olive cultivar FS-17 to *Alternaria alternata* in Southern Spain. *Plant Dis* 92:1252 (2008)
30. Moral, J. Oliveira, R. Trapero, A. Elucidation of the disease cycle of olive anthracnose caused by *Colletotrichum acutatum*. *Phytopathology.* **99**: 548–556 (2009)
31. Noormohammadi, Z. Hosseini-Mazinan, M. Trujillo, I. Rallo, L. Identification and classification of main Iranian olive cultivars using microsatellite markers. *HortSci* 42:1545–1550 (2007)
32. Oliveira, R. Moral, J. Bouhmidi, K. Trapero, A. Caracterización morfològica y cultural de aislados de *Colletotrichum* spp. causantes de la antracnosis del olivo. *Boletín de Sanidad Vegetal Plagas* **31**: 531-548 (2005)
33. Omrani-Sabbaghi, A. Shahriari, M. Falahati-Anbaran, M. Mohammadi, S.A. Nankali, A. Mardi, M. Ghareyazie, B. Microsatellite markers based assessment of genetic diversity in Iranian olive (*Olea europaea* L.) collections. *Sci Hortic-Amsterdam.* **112**: 439 (2007)
34. Palumbo, J.D. Baker, J.L. Mahoney, N.E. Isolation of bacterial antagonists of *Aspergillus flavus* almonds. *Microbial Ecol.* **52(1)**: 45- 52 (2006)
35. Patumi, M. Terenziani, S. Ridolfi, M. Fontanazza, G. Effect of fruit stoning on olive oil quality. *Jaocs.* **80**: 249–255 (2003)



36. Rhouma, A. Triki, M.A. Msallem, M. First report of olive anthracnose caused by *Colletotrichum gloeosporioides* in Tunisia. *Phytopathol. Mediterr.* **49**: 95-98 (2010)
37. Sadeghi, H. Management of olive orchards. Agricultural and Natural Resources Research Center of Sari University. **1**: 242–250 (2010)
38. Sanei, S. J. and Razavi, S. E. Survey of Olive Fungal Disease in North of Iran. *Annual Review & Research in Biology*, **2(1)**: 27-36 (2012)
39. Sergeeva, V. Alves, A. Phillips, A.J.L. *Neofusicoccum luteum* associated with leaf necrosis and fruit rot of olives in New South Wales, Australia. *Phytopathol Mediterr* **48**: 294–298 (2009)
40. Sergeeva, V. Braun, U. Spooner-Hart, R. and Nair, N. First report of *Pseudocercospora cladosporioides* on olive (*Olea europaea*) berries in Australia. *Australian Plant disease Notes*, **3(24)**: (2008)
41. Sergeeva, V. Spooner-Hart, R. and Nair, N. G. First report of *Colletotrichum acutatum* and *C. gloeosporioides* causing leaf spots of olives (*Olea europaea*) in Australia. *Australasian Plant Disease Notes*, **3**: 143–144 (2008a)
42. Singh, R.S. Prashar, M. Post-harvest spoilage of peach and plum fruits in north india due to *Aspergillus*. *Journal of Research Punjab Agricultural University*, **26**: 62-64 (1989)
43. Stark, A.H. Madar, Z. Olive oil as a functional food: epidemiology and nutritional approaches. *Nutr. Rev.* **60**: 170–176 (2002)
44. Talhinhos, P. Sreenivasaprasad, S. Neves-Martin, J. Oliveira, H. Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. *Applied and Environmental Microbiology*, **71**: 2987–2998 (2005)
45. Tjamos, E.C. Graniti, A. Smith, I.M. Lamberti, F. Conference on olive diseases. *EPPO Bulletin*, **23**: 365–550 (1993)
46. Torbati, M. Arzanloua, M. Azadmard-damirchi, S. Babai-ahari, A. Alijani, S. Effect of fungal species involved in the olive fruit rot on the qualitative properties of olive oil. *Archives of Phytopathology and Plant Protection*. **47(3)**: 292–297 (2014)
47. Visioli, F. Galli, C. Natural antioxidants and prevention of coronary heart disease: the potential role of olive oil and its minor constituents. *Nutr. Metab. Cardio. Dis.* **5**: 306–314 (1995)
48. Wilson, D.M. Payne, G.A. Factors affecting *Aspergillus flavus* group infection and aflatoxins. Hyman Health, Veterinary, and agricultural significance (Eaton, D.L. Groopman, J.D. San Diego: Academic Press) (1994)
49. Yekeler, H. Bitmis, K. Ozçelik, N. Doymaz, M.Z. Calta, M. Analysis of toxic effects of *Alternaria* toxins on esophagus of mice by light and electron microscopy. *Toxicol Pathol.* **29**: 492–497 (2001)
50. Zohary, D. Hopf, M. Domestication of plants in the Old World: the origin and spread of cultivated plants in west Asia, Europe, and the Nile Valley, 2nd edn. Clarendon, Oxford, 137–442 (1994)