Plant Cell Biotechnology and Molecular Biology 20(23&24):1180–1188; 2019 ISSN: 0972-2025

FUNGI ASSOCIATED WITH SAFFRON (*Crocus sativus*) IN MOROCCO

I. EL AYMANI, S. QOSTAL, N. MOUDEN, K. SELMAOUI, A. OUAZZANI TOUHAMI, R. BENKIRANE AND A. DOUIRA*

Laboratory of Botany, Biotechnology and Plant Protection, Department of Biology, Faculty of Sciences, Ibn Tofail University, BP. 133, Kenitra, Morocco [IEA, SQ, NM, KS, AOT, RB, AD].

[*For Correspondence: E-mail: douiraallal@gmail.com]

Received: 12 October 2019
Accepted: 20 December 2019
Published: 27 December 2019

Original Research Article

ABSTRACT

In Morocco, saffron is grown in the areas of Taliouine (provine of Taroudante) and Taznakht (province of Ouarzazate). Studies on fungi related to this crop are still very rare. Mycological studies on mycoflora associated with Saffron (*Crocus sativus*) from the main producing area, located in the Taliouine region was carried out between September (corms for cultivation) and December (soil and roots of saffron plants), 2018. The samples (corms and roots with symptoms) were cut up in fragments and disinfected with alcohol. Then, these fragments were cultured on Potato Sucrose Agar and incubated in the growth chamber for 7 days at 28°C. The pure cultures obtained were identified morphologically and microscopically. The fungal complex identified in the soils, corms and roots of saffron plants was almost identical, including *Fusarium solani*, *F. oxysporum*, *F. culmorum*, *F. roseum*, *Fusarium* sp., *Aspergillus fumigatus*, *A. niger*, *Trichoderma* sp., *Rhizopus oryzae* and *Penicillium* sp. Species of the genus *Fusarium,* considered as true pathogens of different crops, are the most represented, with isolation percentages ranging from 6 to 20%. *Trichoderma* sp., a biocontrol agent against various pathogens, has also been isolated from the soils, corms and roots of saffron plants, with a percentage of isolation ranging from 8 to 13%. This is the first time that the species of the genus *Fusarium (F. solani, F. culmorum* and *F. roseum)* and *Trichoderma* have been isolated in Morocco from *Crocus sativus*.

Keywords: *Crocus sativus*; Morocco; corms; roots; symptoms; soils; mycoflora.

INTRODUCTION

Saffron known as *Crocus sativus*, is a medicinally important plant belonging to the family Iridaceae of the order Asparagales. It is widely cultivated in several countries, such as Iran, Italy, Spain, Morocco, France, Greece, China, India and Mexico [1]. The valuable parts of saffron are stigmas and corms. Stigmas represent one of the most expensive spices in the world [2]. Apart from its aromatic and flavoring properties and its use as a colorant for foodstuffs [3], this spice possesses medicinal uses and pharmacological properties [4,5]. As for corms, it insures vegetative propagation of saffron plant [6] whose low multiplication rates and fungal infestation are the major hindrances for availability of sufficient quality planting material [7]. However, as subterranean organs, these corms are constantly prone to diseases caused by fungi [8,9,10], bacteria [11], plant-parasitic nematodes [12] and viruses [13]. Indeed, many phyto-pathogens mainly fungi have been reported in India, Italy and Spain on saffron plants. Among them, *Fusarium* species as *F. oxysporum* [14,15,16], *F. solani* and *F. moniliforme* [17],

Sclerotium rolfsii [18], *Rhizoctonia solani* [19], *Macrophomina phaseolina* [20,21], and *Penicillium aurantiogriseum* Dierckx (*Penicillium cyclopium* Westl.), and *Penicillium hirsutum* Dierckx (*Penicillium corymbiferum* Westl.) [22,23]. Indeed, most of pathogenic species were isolated from corms which affect post-development of this organ and in turn disturb saffron viability, propagation and yield [9]. Also, Rubio-Moraga et al. [24] have reported in Italy several species associated with saffron corms belonging to genera of *Fusarium, Rhizoctonia, Penicillium, Aspergillus, Sclerotium, Phoma, Stromatinia, Cochliobolus* and *Rhizopus*. These species, cases of *Fusarium, Sclerotium* and *Rhizoctonia*, are pathogenic on corms and saffron plants.

In Morocco, saffron cultivation is an important economic activity in the mountainous regions of Taliouine (Taroudant province) and Taznakht (Ouarzazate province) where it covers an area of 105 ha [25]. However, up to date, little is known about the phytosanitary status of this perennial crop culture and few studies on composition of fungal community associated with saffron plants were realized. Hence, the main purpose of this work is the isolation and identification of fungal flora related to saffron plants growing in two Morocco's saffron producing regions.

MATERIALS AND METHODS

Surveys and Sampling

The samples of soil, corms and roots of saffron plants growing in saffron culture of the Taliouine region, Ouarzazate province (Fig. 1), aged of 4 years (plots of saffron culture exploited during 4 years), were collected between September and December during 2018.

Fig. 1. Safran's production zone in Morocco

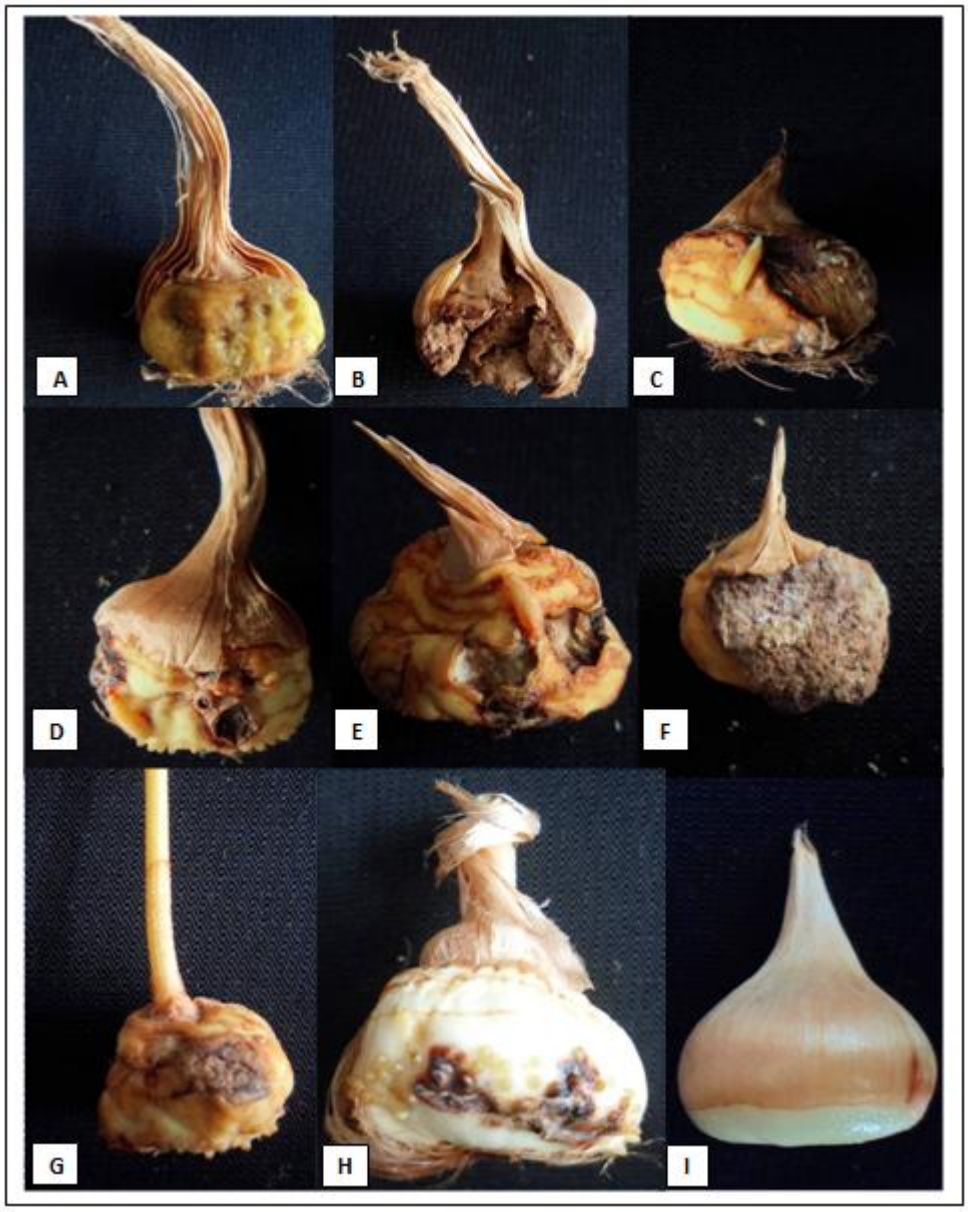

Fig. 2. Symptoms of corm rots in naturally diseased saffron corms collected from farms. Rotten bulb of *Crocus sativus* showing reduced volume and diameter with discoloured sections (A, B, C, D, E, F, G, H); (I) Healthy corm

Diseased corm (Fig. 2) and plant samples were collected and brought to laboratory for mycological analysis. Symptomatic and symptomless corms were cut up in fragments of 1 cm lentgh, 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), supplemented with 50 mg/1 of chloramphenicol, and incubated in the growth chamber for 7 days on darkness at 28°C. Some developed colonies were then observed for the species determination. Others were transferred to

P.S.A medium and incubated under the same conditions for 7 days.

Root samples were separated from individual plants. Each root sample was washed thoroughly under running tap water to remove soil particles, dried and cut into 1 cm length segments. The root segments were disinfected for 10 minutes with bleach diluted to 10%, rinsed three times with sterile distilled water and dried on sterile blotting paper. They were placed in 10 mm diameter Petri dishes containing 15 ml of PSA medium (Potato Sucrose Agar) supplemented with 50 mg. The cultures were incubated in the growth chamber for one week under a photoperiod of 16 h at 26°C.

The determination of the fungal community associated with soil was carried out according to soil plate's technique [26]. Each culture soil was dried at 30°C and grounded in a sterilized mortar. A quantity of 5 to 15 mg was placed on the PSA medium, humidified by one or two drops of sterile distilled water and spread out on the medium. Thereafter, the PSA culture medium containing 30 mg Penicillin and 50 mg of chloramphenicol is poured onto this dispersed soil [27]. The Petri dishes gently shacked before solidification of the medium were incubated in the growth chamber at 28°C and in the dark. Successive transplants are used to purify isolated fungal species.

The observation of different cultures and fragments under the optical microscope has allowed us to identify the fungal species by using the identification keys of Gilman [28], Tarr [29], Ellis [30], Chidambaram et al. [31], Domsch et al. [32] and Champion [33].

The percentage of isolation of different fungal species is calculated according to the method of Champion [33] which defines the frequency of isolation of different fungi from 100 lesions or 100 root rot present on the plants studied according to the equation:

$$PI = NsPx / NT \times 100$$

NsPx: Number of segments containing the fungal specie x.

NT: Total number of segments used in the isolation.

Analysis of the variance and of the mean comparisons using the LSD test (p = 5%) were performed using the software STATISTICA program. The percentages were transformed into Arcsin XP (where P is the proportion of percentage).

RESULTS

The examination of corm samples showed various infection levels. They were severely affected and exhibit rot development, lesions and extensive necrosis (Fig. 1). On the basis of cultural, microscopic and morphological characteristics, ten fungal species were isolated and identified (Figs. 3, 4, 5). It was also noticed that all corm, root and soil samples harboured approximately identical fungal community (Table 1). The main fungal taxa identified include *Fusarium* with five species *Fusarium solani, F. oxysporum, F. culmorum, F. roseum* and *Fusarium* sp., *Aspergillus* with two species *Aspergillus fumigatus* and *A. niger,* in addition to *Trichoderma* sp., *Rhizopus oryzae* and *Penicillium* sp. (Table 1). The symptoms are not specific; two to four fungal species can be isolated from the same corms or from the roots of the same plant.

Fusarium solani and *F. oxysporum* occurred in soil, corms and roots of saffron plants with isolation frequencies in the order of 13 - 6%, 11 - 16% and 20 - 16% respectively. *F. culmorum* and *Fusarium* sp. were isolated from soil at respective percentages of 6% and 10%, whereas, *F. roseum* was detected in soil and roots of saffron plants with isolation percentages of 6 and 25% respectively.

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

Moreover, the results given in Table 1 indicated that the occurrence of *Aspergillus fumigatus* and *A. niger* differed significantly. *A. fumigatus* was recovered at frequency of 13% in soil, 14% in corms and 10% in roots compared with frequencies ranged from 2% to 3% for *A. niger* in rhizosphere soil, corms and roots of saffron plants. Similarly, the isolation percentages of *Penicillium* sp. varied from 10% to 14%, those of *Rhizopus oryzae* from 8 to 11%. *Trichoderma* sp. has been isolated at the level of soil, corms and roots of

saffron plants reaching percentages varying between 8% and 13%. Furthermore, it was found significant isolation percentages ranged from 16% and 27% characterizing an undetermined species assemblage presents in different soil, corms and roots of saffron plants samples.

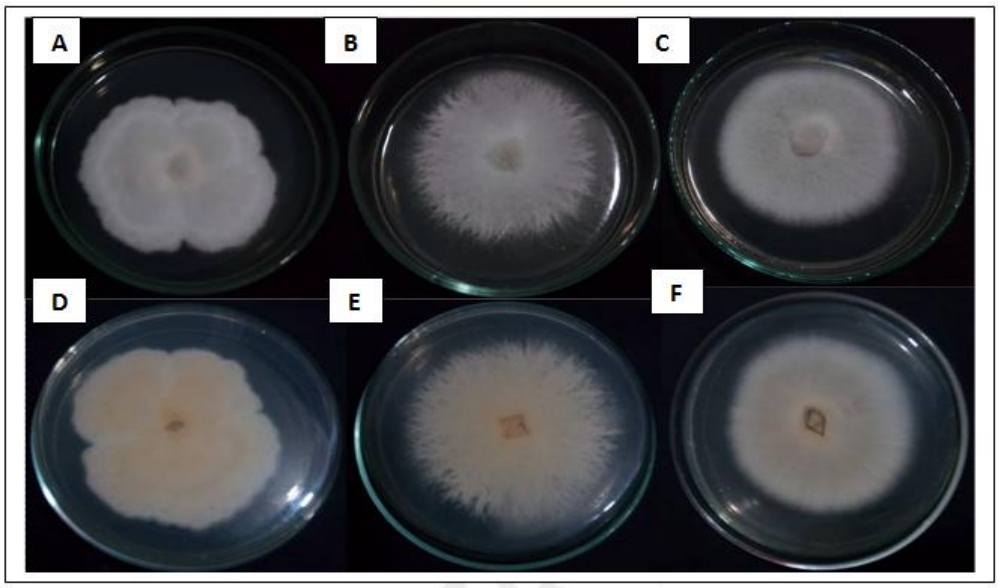

Fig. 3. Colony features on PSA of three species of *Fusaruim* recovered from soils, corms, roots of *Crocus sativus*. *Fusarium roseum:* plate top, reverse A and D*; F. oxysporum:* plate top, reverse B and E; *F. solani*: plate top, reverse C and F

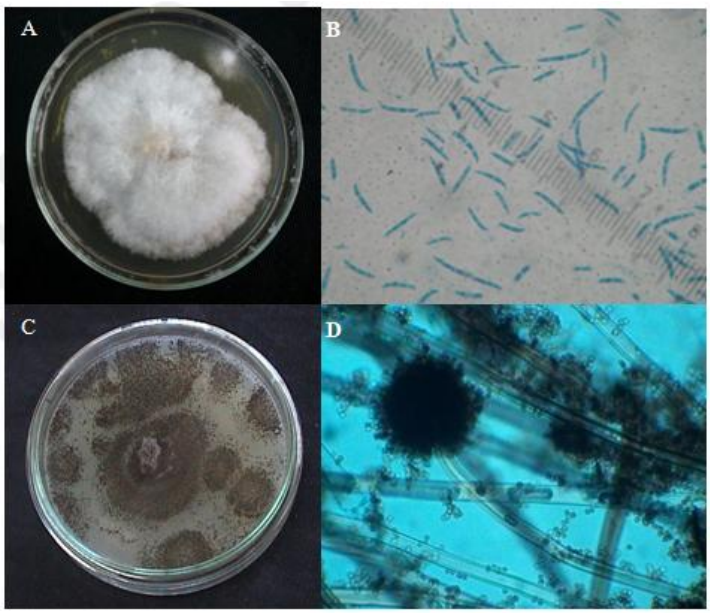

Fig. 4. *Fusarium oxysporum* and *Aspergillus niger* colony features on PSA: (A and C), microscopic examination of macroconidia of *Fusarium oxysporum* (B) and sporangium of *Aspergillus niger* (D)

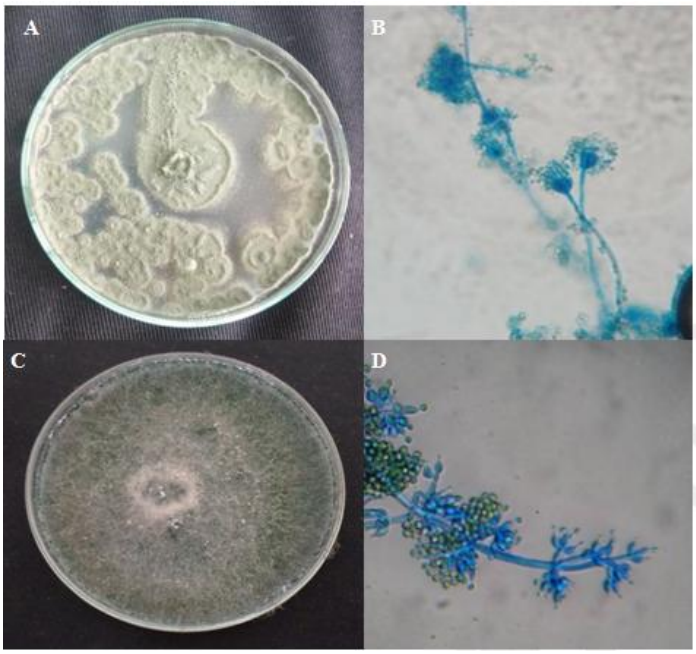

Fig. 5. *Penicillium* sp. and *Trichoderma* sp. colony features on PSA (A and C). Microscopic examination of brush arrangement of *Penicillium* sp. conidia (B), conidia and phialides of *Trichoderma* sp.(B and D)

Table 1. Isolation percentages of fungal species recovered from soils, corms and roots of saffron plants growing in the Taliouine site

Soils		Corms		Roots	
Fungal species	**PI %**	**Fungal Espèce**	**PI %**	**Fungal species**	**PI %**
Fusarium solani	13^{ab}	*Fusarium solani*	11^{b}	*Fusarium solani*	20^{a}
Fusarium oxysporum	6^{c}	*Fusarium oxysporum*	16^{b}	*Fusarium oxysporum*	16^{ab}
Fusarium culmorum	6^{c}	*Fusarium culmorum*	-	*Fusarium culmorum*	-
Fusarium roseum	6^{c}	*Fusarium roseum*	-	*Fusarium roseum*	25^{a}
Fusarium sp.	10^{b}	*Fusarium* sp.	-	*Fusarium* sp.	-
Trichoderma sp.	13^{ab}	*Trichoderma* sp.	11^{b}	*Trichoderma* sp.	8^{c}
Aspergillus fumigatus	13^{ab}	*Aspergillus fumigatus*	14^{b}	*Aspergillus fumigatus*	10^{b}
Aspergillus niger	2^{d}	*Aspergillus niger*	3^{c}	*Aspergillus niger*	2^{d}
Penicillium sp.	14^{a}	*Penicillium* sp.	10^{b}	*Penicillium* sp.	12^{b}
Rhizopus oryzae	10^{b}	*Rhizopus oryzae*	11^{b}	*Rhizopus oryzae*.	8^{c}
undetermined	16^{a}	undetermined	27^{a}	undetermined	16^{ab}

PI (%), isolation percentage

DISCUSSION

The present study showed detection of ten fungal species which colonizes soil, corms and roots of saffron plants. *Fusarium* genus was the most represented with 5 species. To our knowdledge, this is the first study indicating presence of *F. oxysporum* and *F. solani* in soils and sampled saffron plant parts. Previous results support this findings and *F. oxysporum* which incited corm rots was reported by many research studies [8,34,35,24,10]. According to Brayford et al. [36], *F. oxysporum* survives in infected corms and in the soil as mycelium, chlamydospores, macro-conidia and microconidia. Similarly, Wani [37] detected *F. solani* and *F. oxysporum* among fungal

complex responsible of frequent occurrence of saffron corm rot. In contrary to previous studies signaling isolation of *F. moniliforme* and *F. equiseti* from infected saffron corms [38,37], *Fusarium* species like *F. culmorum* and *F. roseum* have not been reported as pathogens or endophytes from saffron plants so far. In Morocco, these species isolated from Moroccan soils were responsible of wheat and barley crown rots [39,40,41,42]. In India, *Rhizoctonia*, *Phytophthora*, *Phialophora mustea* and *Cadophora malorum* have been reported as prevalent endophytes identified on saffron corms and stems [43,44]. Additionally, *Rhizoctonia violacea* and *Fusarium oxysporum* have been proved to be the causal organisms associated to root rot disease or death of saffron plants in Morocco [45].

Similarly, Chamkhi et al. [46] reported that the roots of saffron plants collected in Morocco (Taliouine site) are colonized by *Rhizopus oryzae, Aspergillus fumigatiaffinis* and *Aspergillus niger*.

Moreover, isolation of *Trichoderma* sp. from soils, corms and roots of Moroccan saffron cultivation areas is a positive result. Indeed, this competitive antagonist, recognized as an endophyte fungus of roots but also of aerial plant tissues, including trunks, stems and fruits of different plant species [47,48], can be used as an antagonist of corm-borne pathogens.

CONCLUSION

Fungi isolated from soil of saffron plantations, corms or roots of saffron form a diversified fungal complex. Therefore, it seems difficult to know precisely the etiology of these species. Hence, pathogenicity testing on saffron corms will be necessary to determine the pathogenic or saprophytic ones. Also, it is important to know which one is able to affect corms and greatly influence the development of saffron plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Giorgi A, Scheurer T. Alpine resources: Assets for a promising future Conclusions from the Forum Alpinum 2014. Mountain Research and Development. 2015;35(4): 414-415.
2. Busconi M, Colli L, Sánchez RA, Santaella M, Pascual MDLM, Santana O, Roldán M, Fernández JA. AFLP and MS-AFLP analysis of the variation within saffron crocus (*Crocus sativus* L.) Germplasm. 2015;10(4).
   DOI: 10.1371/journal. pone.0123434
3. Babaei N, Salamci MU. State Dependent Riccati Equation Based Model Reference Adaptive Stabilization of Nonlinear Systems with Application to Cancer Treatment. World Congress. The International Federation of Automatic Control Cape Town, South Africa. 2014; P265-270.
4. Hadizadeh F, Mohajeri SA, Seifi M. Extraction and purification of crocin from saffron stigmas employing a simple and efficient crystallization method. Pakistan Journal of Biological Sciences. 2010;13(14):691-698.
5. Bhargava VK. Medicinal uses and pharmacological properties of *Crocus sativus* Linn. (Saffron). International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3(3):22-26.
6. Vurdu H, Guney K, Cicek F. Biology of *Crocus olivieri* sub *spolivieri*. Acta Horticultural. 2004;650:71-85.
7. Kiran D, Madhu S, Markandey S, Paramvir SA. *In vitro* cormlet production and growth evaluation under greenhouse conditions in saffron (*Crocus sativus* L.)– A commercially important crop. Life Sci. 2011;11(1):1-6.
8. Cappelli C. Occurrence of *Fusarium oxysporum* f. sp. *gladioli* on saffron in Italy. Phytopathol. Mediterr. 1994;33(1):93–94.
9. Ahrazem O, Trapero A, Gómez D, Rubio-Moraga A, Gómez GL.. Genomic analysis and gene structure of the plant carotenoid dioxygenase 4 family: A deeper study in *Crocus sativus* and its allies. Genomics. 2010;96(4):239-250.

10. Palmero D, Rubio-Moraga A, Galvez-Patón L, Nogueras J, Abato C, Gómez-Gómez L, Ahrazem O. Pathogenicity and genetic diversity of *Fusarium oxysporum* isolates from corms of *Crocus sativus.* Industrial Crops and Products. 2014;61: 186–192.
11. Fiori M, Ligios V, Schiaffino A. Identification and characterization of *Burkholderia* isolates obtained from bacterial rot of saffron (*Crocus sativus* L.) grown in Italy. Phytopathol. Mediterr. 2011;50(3):450−461.
12. Mokrini F, Laasli SE, Karra Y, El-Aissami A, Dababat AA. Diversity and incidence of plant-parasitic nematodes associated with saffron (*Crocus sativus* L.) in Morocco and their relationship with soil physicochemical properties. Nematology. 2019;1-16.
13. Chen J, Chen JS. Occurrence and control of mosaic disease (Turnip mosaic virus) in saffron (*Crocus sativus*). Zhejiang Nongye Kexue. 2000;3:132–135.
14. McDonald BA. The population genetics of fungi: Tools and techniques. Phytopathology. 1997;87(4):448-453.
15. Cappelli C, Di-Minco G. Results of a triennial study on saffron diseases in Abruzzi. Inf. Fitopatol. 1999;49:27–32.
16. Bashir S, Mughal MN, Nehvi FA, Dar SA, Un-nissa S, Dar ZA, Hakeem SA, Wani RA, Baba JA, Habib M. Identification of sources of resistance in Saffron (*Crocus sativus* L.) to *Fusarium oxysporum* causing corm rot disease. Int. J. Curr. Microbiol. App. Sci. 2017;6(11):3660-3665.
17. Jan J, Baba SA. Corm rot of saffron: Symptoms and biological management. EC Microbiology. 2018;14(1):01.
18. Kalha CS, Gupta V, Gupta D, Priya S. First report of sclerotial rot of saffron caused by *Sclerotium rolfsii* in India. Plant Disease. 2007;91(9):1203-1203.
19. Mir GH, Devi LS. Saffron corm rot and their management. Proceedings of the National Symposium on detection and management of plant diseases using conventional and modern tools and IPS Zonal Meeting (MEZ), Luchknow. 2004; 20-20.
20. Thakur RN, Singh C, Kaul BL. First report of corm rots in *Crocus sativus*. Indian Phytopathology. 1992;45(2):278.
21. Ionita A, Lliescu H, Jinga V, Iordache E. *Macrophomona phaseolina* dangerous parasite of cropped plants possibilities for control. Problem de-protectia Plantelor. 1995;23(2):179–196.
22. Gu ZF, Zhi Y. A study on biological characteristics of *Penicillium corymbiferum* on *Crocus.* Acta Agriculturae Shanghai. 1997;13(4):71–74.
23. Fiori M. Avversità. In: *Zafferano*. *Storia, Cultura, Coltivazione e Impiego a San Gavino Monreale e in Sardegna*. (F. Sanna, ed.) E.R.S.A.T. Centro Zonale di Guspini. Medio Campidano, Sardinia, Italy. 2002; 68–73.
24. Rubio-Moraga Á, Gómez-Gómez L, Trapero A, Castro-Díaz N, Ahrazem O. Saffron corm as a natural source of fungicides: The role of saponins in the underground. Industrial Crops and Products. 2013;49:915-921.
25. Aboudrare A, Aw-Hassan A, Lybbert TJ. Importance socio-économique du Safran pour les ménages des zones de montagne de la région de Taliouine-Taznakht au Maroc. Rev. Mar. Sci. Agron. Vét. 2014;2:5–14.
26. Warcup JH. The soil plate method for isolation of fungi from soil. Nature. 1950;166:117-118.
27. Rapilly F. Les techniques de mycologie en pathologie végétale. Ann. Epiphyt. Institut National de la Recherche Agronomique. 1968;19:101.
28. Gilman CJ. A manual of soil fungi, Second Edition. The Iowa State college Press Ames, Iowa, U.S.A. 1957;452.
29. Tarr SAJ. Diseases of sorghum, sudan grass and broom corn. Commonwealth Mycological Institute, Kew, England. 1962; 380.
30. Ellis MB. Dematacеous Hyphomycetes. Common wealth Mycological Institute Kew, Surrey, England. 1971;608.
31. Chidambaram P, Mathur SB, Neergaard P. Identification of seed-borne *Drechslera* species. Handbook on Seed Health Testing, series 2 B. 1974;3:165-207.

32. Domsch KH, Gams W, Anderson TH. Compendium of soil fungi. Academic Press, London. 1980;1:859.
33. Champion R. Identifier les champignons transmis par les semences. INRA, Paris. 1997;398.
34. Di Primo P, Cappelli C. Preliminary study on vegetative compatibility groups of *Fusarium oxysporum* f. sp. *Gladioli* isolates obtained from saffron in Italy. Informatore Fitopatologico. 2000;50:35–38 (in Italian).
35. Primo DP, Cappelli C, Katan T. Vegetative compatibility grouping of *Fusarium oxysporum* f. sp. *gladioli* from saffron. European Journal of Plant Pathology. 2002;108:869–875.
36. Brayford D. *Fusarium oxysporum* f. sp. *gladioli*. Mycopathologia. 1996;133:47–48.
37. Wani A. Studies on corm rot of saffron (*Crocus sativus* L.). PhD thesis, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India. 2004;108.
38. Dhar AK. Bio-ecology and control of corm rot of saffron (*Crocus sativa* L) Master's thesis Division of Plant Pathology, SKUAST-K (J & K). 1992;109.
39. El Yousfi B. Contribution à l'étude de l'étiologie de l'épidémiologie et des pertes en rendement dues aux pourritures racinaires du blé et de l'orge. Mémoire de 3ème cycle agronomique, I.A.V Hassan II, Rabat, Maroc. 1984;77.
40. Cromey MG, Parkes R A, Fraser PM. Factors associated with stem base and root diseases of New Zealand wheat and barley crops. Australian Plant Pathol. 2006;35: 391–400.
41. Paulitz TC, Schroeder KL, Schillinger WF. Soilborne pathogens of cereals in an irrigated cropping system: Effects of tillage, residue management, and crop rotation. Plant Disease. 2010;94(1):61-68.
42. Qostal S, Kribel S, Chliyeh M, Serghat S, Selmaoui S, Ouazzani Touhami A, Zaarati H, Benkirane R, Douira A. Study of the fungal complex responsible for root rot of wheat and barley in the north-west of Morocco. Plant Archives. 2019;19(2): 2143-2157.
43. Raj P, Khan SS, Modak M, Lone ZA, Rather SA, Yaqoob M. Biodiversity of endophytic fungi in saffron (*Crocus sativus*) and antimicrobial activity of their crude extract. Indo Am J Pharm Res. 2013;3:3702-3713.
44. Wani ZA, Mira DN, Arora P, Riyaz-UI-Hassan S. Molecular phylogeny, diversity, community structure, and plant growth promoting properties of fungal endophytes associated with the corms of saffron plants: An insight into the microbiome of *Crocus sativus* Linn. Fungal Biol. 2016;120(12): 1509-1524.
45. Bentata F, Lage M, Bakhy K, Ibrahimi M, Jbair A, El Aissami A, Kissayi KH, Labhilili M. Sanitary assessment of saffron corms and soil from Great Moroccan production areas: Taliouine and Taznakht. Acta Hortic. 2017;1184:263-266.
46. Chamkhi I, Sbabou L, Aurag J. Endophytic Fungi Isolated from *Crocus sativus* L. (Saffron) as a Source of Bioactive Secondary Metabolites. Pharmacognosy Journal. 2018;10(6):1143-1148.
47. Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Samuels GJ, Choi I-Y, Holmes KA. Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. Planta. 2006;224(6):1449-1464.
48. Bailey BA, Bae H, Strem MD, Crozier J, Thomas SE, Samuels GJ, Vinyard BT, Holmes KA. Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma* cacao. Biol. Control. 2008;46(1):24-35.