Pure Appl. Biol.,14(2):249-258,June, 2025
http://dx.doi.org/10.19045/bspab.2025.140023

Research Article

Identification and pathogenicity of *Fusarium* species isolated from maize seeds collected from the district Sialkot

Andliba Batool[1], Brian Gagosh Nayyar[1*], Akasha Naseer[1], Ayesha Sarwar[1], Noshaba Olikh[1] and Wajiha Seerat[2]
1. Department of Biological Sciences, University of Sialkot, Sialkot 51310, Pakistan
2. Department of Botany, PMAS-Arid Agriculture University, Rawalpindi 46300, Pakistan
**Corresponding author's email: brian_gagosh@hotmail.com*

Citation
Andliba Batool, Brian Gagosh Nayyar, Akasha Naseer, Ayesha Sarwar, Noshaba Olikh and Wajiha Seerat. Identification and pathogenicity of Fusarium species isolated from maize seeds collected from the district Sialkot. Pure and Applied Biology. Vol. 14, Issue 2, pp249-258. http://dx.doi.org/10.19045/bspab.2025.140023

Received: 06/08/2024 Revised: 05/10/2024 Accepted: 09/10/2024 Online First: 19/10/2024

Online first accepted article

Abstract

Fusarium species significantly affect the quality and marketability of maize (*Zea mays* L.), which are responsible for quality and quantity losses on a commercial scale. This study aimed to identify morphologically and check the pathogenicity of *Fusarium* species isolated from maize seeds widely grown in the district Sialkot. A total of 25 maize kernel samples were obtained from five tehsils of district Sialkot and subjected to the isolation of *Fusarium* species by agar plate method, blotting paper method, and deep-freezing methods from both surface disinfected seeds and non-disinfected seeds. About five *Fusarium* species viz, *Fusarium oxysporum* (F1)*, Fusarium solani* (F2), *Fusarium verticillioides* (F3)*, Fusarium culmorum* (F4), and *Fusarium graminearum* (F5), were isolated based on morphological identification. Whereas the most frequent isolate was identified as F1. Its isolate was more pathogenic than F2 and control. Moreover, its cultural filtrate also showed the lowest germination (10%) and vigor index (5) of maize seedlings. The results of this study indicate that the most significant method for the isolation of *Fusarium* was the agar plate method, which showed the highest infection rate of 2 – 15 % from samples collected from tehsil Pasrur. This isolation and morphological identification of the linked *Fusarium* with maize will be helpful for the molecular identification of *Fusarium* species in future research. For sustainable agriculture, this study will aid in controlling seed-borne pathogens to minimize crop yield losses.

Keywords: *Fusarium oxysporum*; Maize; Morphological Identification; Pathogenicity

Introduction

Maize is considered the third significant cereal crop in Pakistan [1]. Maize grains contain important nutrients such as starch, protein, oil, fiber, sugar, and ash in the amount of 72%, 10%, 4.8%, 5.8%, 3%, and 1.7%, respectively [2]. Maize may also be managed into diverse diets and industrial foods, such as beverages, industrial alcohol, etc. [3]. The worrying population growth rate in many developing countries like Pakistan has increased many issues, such as food security issues [4].

Many weeds, pests, and pathogens have a great effect on crop production and its quality

[5]. The whole maize plant is mostly contaminated by fungal species at the pre-harvest and post-harvest stages. The contaminated fungal species can produce chemicals and mycotoxins that can impose carcinogenic effects on both livestock and animals [6].
Many fungi can cause diseases among maize plants, but it is observed that *Fusarium* species are among the most familiar pathogenic communities of fungi that affect maize crops and result in disease developments such as *Fusarium* ear rot (FER) [7]. The *Fusarium* genus of fungi is a significant pathogen, leading to great yield losses throughout the world [8]. The *Fusarium* occupies fourth and fifth place among the top ten fungal pathogens [9]. *Fusarium* species cause severe maize plant diseases, which ultimately could affect the production of maize and its quality; it can lead to big economic loss as well as mycotoxins contaminated kernels, causing serious diseases in humans and animals [10]. Most of the *Fusarium* species cause ear rot diseases and stalk rot of maize disease in European countries. Moreover, red ear rot in maize plants occurs due to *Fusarium graminearum* [11]. The major threat to the production of maize is ear rot initiated by *Fusarium* spp. and the ability to produce mycotoxins that have potential health dangers for consumers. Maize seeds associated with the *Fusarium* are mostly contaminated with the mycotoxin fumonisins, which may cause cancer in human beings. It showed a direct link between the consumption of maize and cancer of the esophagus [12]. The mycotoxins mostly produced by pathogenic *fusarium* species are moniliformin, fumonisins, zearalenone, trichothecenes, and deoxynivalenol [13]. Mycotoxins are frequently formed and accumulate in afflicted tissues, and contaminations of maize with phytopathogenic and toxigenic *Fusarium* species can induce illnesses in vegetative and generative organs [14].
To the best of our knowledge, identification and pathogenicity of *Fusarium* species in maize seeds have not been conducted in the Sialkot region, and this research aimed to isolate and identify the associated *Fusarium* species from maize seed and examine the effect of isolated *Fusarium* on the germination of seed, growth, and the persistence of maize in the Sialkot region.

Material and Methods

Collection of samples

Five infected cobs from five maize-producing tehsils of the Sialkot district were collected at the mature stage of the maize plant and brought to the laboratory in the Department of Biological Sciences, University of Sialkot. These samples were shade-dried and subjected to the isolation of *Fusarium* species and its pathogenicity test. All the samples were kept in the refrigerator at 4ºC until used.

Isolation and identification of maize seed-borne fungi

The fungi were isolated by agar plate, blotter paper, and deep-freezing methods by following the standard protocols of the International Seed Testing Association (ISTA) [15]. Seeds were surface sterilized with 2% aqueous sodium hypochlorite (NaOCl) for 5 min and then washed with distilled water. Sixty sterilized and unsterilized seeds were used in all methods for the isolation of fungi. In the agar plate method, Potato dextrose agar (PDA) containing 4 g of potato starch, 20 g of glucose, and 15 g of agar (Oxoid, Basingstoke, UK) was prepared by adding 39 g of dehydrated PDA to 1 L of double-distilled water and autoclaved. Following autoclaving at 121ºC and 15 lb/in^2 for 20 min, the PDA was cooled to 50ºC, 100 mg/L streptomycin was added, and the final medium was poured into 9-cm-diameter pre-sterilized Petri dishes. On each dish, 10

Online first accepted article

surface sterilized (2% NaOCl) and unsterilized maize seeds were equally spaced and incubated at 25℃ with an alternating light-dark cycle in a growth chamber (Sanyo, Moriguchi, Japan) with a fluorescent light (Sylvania, Erlangen, Germany). For blotter and deep-freezing methods, the treated and untreated seeds were placed on three layers of moistened blotters placed in Petri dishes at the rate of 10 seeds per plate. The plates were incubated at 25 ± 1 °C for seven days except in the deep-freezing method in which the plates were incubated for 24 hours at 25 ± 1 °C and then transferred to -20°C in a freezer for 24 hours, followed by incubation at 25 ± 1 °C for 7 days. Isolated fungi were examined and identified periodically according to published procedures to confirm identity [16].

The rate of growth of *Fusarium* strains and a colony of cultures was kept in an incubator and incubated for seven days in the dark and was examined on PDA. Apparent characteristics such as shape and size of conidia were noted from *Fusarium* strains in 9 – 15 days. After 4 weeks, polyphialides and expansion of sporodochia were also observed. The other microscopic characteristics, such as the texture of the surface and the color of the colony were also identified [16].

The apparent properties were recorded, and the frequency of isolation (Fr) and also relative density (RD) of fungi were measured through the given formula:

$$\text{Fr}\ (\%) = \left(\frac{ns}{N}\right) \times 100 \qquad \text{RD}\ (\%) = \left(\frac{ni}{Ni}\right) \times 100$$

ns = number of samples on which a fungus occurred, N = total number of samples, ni = number of isolates of a given fungal genus/species, Ni = total number of fungal isolates obtained.

Pathogenicity test

The most frequent *Fusarium* isolate was grown on PDA, and their conidia were collected by making a suspension that contained 2% tween 20. The suspension obtained was filtered with a sterilized cheesecloth. Then *Fusarium* spores were adjusted to 1×10^6 conidia per mL by using a hemocytometer [17].

Maize plants were grown in pots with 13 × 11cm size, 3 plants per pot in a sterilized environment (soil, sand, farmyard manure; 1:1:1 w/w/w). An inoculating channel approximately 3 cm deep was formed bearing all seedlings in the soil and also spore suspension filled. On the other hand, controls are given distilled water. Three pots of each treatment inoculated plants and controls were settled. The infected plants were shielded with transparent bags of plastic for one day (24 hr) to maintain suitable moisture content and then put in the greenhouse at the 25/ 20 °C day/night temperatures. Then inoculated plants were watered with the distilled H_2O periodically. After 30 dpi, the disease symptoms in inoculated plants were noted and the data was collected using the disease severity index. Disease indications on roots and stems were counted by a four-point definite disease severity scale: 0¼ no wilting indications, 1¼ 25% wilting, 2¼ 50% wilting, and 3¼ 100% wilting and plant death. Then disease severity (DS) will be calculated by following the formula:

DS = (No. of infected leaves) / (Total no. of leaves in a plant) × 100

To check the inoculated isolates of *Fusarium* initiated the disease symptoms in the maize plantlets, re-isolations were performed from infected parts of maize plants to fulfill Koch’s postulates. After 7 dpi, inoculation-indicative tissues were sterilized in 5% NaOCl two times for 2 min followed by washing and autoclaved purified water for 2 min. Then 3 to 4 pieces (3-6mm in length) of

Online first accepted article

stem or root per plant were removed beneath disease-free regions and transferred to PDA in Petri dishes containing antibiotics. These Petri dishes were placed in incubators at 28 ± 1℃ while waiting for a distinctive look of *Fusarium* colonies approximately after the 5 to 7 days of planting.

Effects of filtrated culture on the germination of seed and seedling growth

The most frequent *Fusarium* isolate ($1x10^6$ conidia per ml) was added into Erlenmeyer flasks (50 ml) containing PDA and then incubated at 28 ℃ for 14 days. After that, the fungal mycelia were gently separated away. In a 50 ml volume flask, ethyl acetate: methanol: chloroform (v/v/v) 3:1:2 was mixed in each flask which already had culture media, monitored by using a rotary shaker overnight. Extracts were centrifuged for 30 minutes at 5000 rpm, and then the supernatant was incubated for 10 h at 45℃ to distillate the extract 10ml volume [18].

Sterilized maize kernels in 5% NaOCl for about 2 minutes, rinsed these seeds in disinfected distilled water and placed in 10 ml culture filtrate. Then, these maize seeds were followed by incubation for 24h at 28 ± 2℃. Then, ten seeds were relocated from the filtrated suspension and then washed in autoclaved distilled H_2O. Agar medium (1.5%) in water and 10 seeds in each petri dish and control seeds were treated with autoclaved distilled water. Lengths of root and shoot were noted after the 7 days of incubation. In accumulation, a vigor index was counted [19] by the succeeding formula: Vigor index = Seed germination (%) × Seedling Length (Shoot + Root Length)

Statistical analysis

All the data was analyzed by using SPSS (version 16.0; SPSS Inc., Chicago, IL), and the mean difference was compared by post hoc test (P<0.05).

Results and Discussion

Morphological identification of *Fusarium* isolates

Two hundred and forty-four (244) isolates of *Fusarium* were isolated from the maize seeds by Agar plate, Blotter paper, and Deep-freezing methods. Above all methods, the agar plate method showed significant results in the highest infection (2 – 15 %) rate as compared to deep freezing and blotter paper methods. In all methods, the most dominant species belonging to the genus *Fusarium* was frequently isolated and recognized as *F. oxysporum* (4.5%), followed by *F. solani* (1.5%), *F. verticillioides* (1.5%) and *F. graminearum* (1.5%) (Table 1).

These isolates are recognized by their colony characters and based on conidial shape. At the start of colonization, the colony color of *F. oxysporum* was white, cottony, and dark purple under the surface. The range of growth rate was 3 – 5 cm in 7 days. The spores were oval to ellipsoid or bearing kidney shape, and their chlamydospores formed in the form of a chain. Conidia were short to medium length, straight to slightly curved, bearing slender shapes with thin walls having size 44-78 × 3.3-5.6µm. The colony color of *F. solani* was white creamy to white greyish, and spores were oval and ellipsoid and mostly present at the aerial mycelia. The chlamydospores are globose to oval in shape, and their macroconidia are relatively wide and straight, with 34.4 – 44 µm. The colony color of *F. verticilliodies* having white mycelia pigmentation may arise from orange to violet-grey. Their conidia may form chains that are relatively long and slender, slightly straight, with a size of 40 – 50 µm. The colony color of *F. culmorum* was different orange-reddish shades or whitish pink to orange cotton and was filamentous in appearance, and their conidia are present in the aerial mycelium. These most frequent isolates were next used for pathogenicity tests (Fig 1). These results were confirmatory

Online first accepted article

with the results of Panchal and Dhale [20]. Leslie and Summerell [16] suggested the isolate's separation into subcategories based on morphological character as initial identification. Niaz and Dawar [1] also reported some *Fusarium* species, including above all species, infecting maize seeds in Pakistan.

Pathogenicity test

When pathogenicity tests of *Fusarium* isolates were performed on maize plants, high disease severity, and disease incidence were observed on plants inoculated with *F. oxysporum,* and no incidence was found in control treatment plants. It shows that *F. oxysporum* has the potential to cause disease in maize, resulting in loss of yield (Table 2). Isolates F1, F2, F3, and F4 were inoculated to maize plants, resulting in a dark brown color of leaves about 14 days after the treatment, plants constantly became weak, started to decline, and plants leaves got dark brown and necrotic. Stem showed stunted growth as compared to healthy plants. The roots of the plant showed reduced growth, shrank, and the whole plant wilted after 30 days of treatment (Fig 2). The statistical data showed that the agar plate method showed significant results ($p < 0.05$) among the applied methods, and maximum infection of fungus was also counted in this method. Again, re-isolation from the infected plants and parts of treated plants was carried out, and it remained successful, the re-isolated fungus was identical to the isolates that were previously used for inoculating maize plants, and here Koch's postulates were fulfilled.

Nayyar *et al*. [21] found the presence of *Fusarium* species from the infected symptomatic parts of the sesame plant, showing similar results to the present work. So, the outcomes recommended that contamination of seed with seed-borne *Fusarium* results in less seed germination. The study of Scauflaire et al. also showed the occurrence of Fusarium species at the stalk, which is an aggressive cause of inoculum for the maize crop, and these research findings agree with the present research results. Pathogenicity analysis of Scauflaire et al. showed disagreeable results with the present work that *F. temperature* can cause sprout distortion and stalk rot under greenhouse circumstances. *F. temperatum* has the potential to cause maize infections and put a latent risk to maize yield and food security [11].

Cultural filtrates effects of Fusarium on germination of maize

The studied isolates, such as F1, F2, F3, and F4, revealed significant differences compared to the control treatment. The germination percentage of maize seeds and vigor index of maize plants were badly affected by cultural filtrate of isolates F1, with a 90% reduction in seed germination and the lowest vigor as compared to control plants and remaining isolates (F2 and F4) (Table 3) (Fig. 3).

Previous reports on the prevalence and pathogenicity of *Fusarium* species relied only on samples taken from symptomatic, infected plants. For instance, several inoculated seedlings died from numerous Fusarium species, including *F. oxysporum*, *F. proliferatum*, and *F. redolent*, which proved to be very virulent on onion. *F. proliferatum*, one of the examined isolates, demonstrated great onion pathogenicity in inoculation testing [22]. Given that this fungus has been implicated in major issues with corn, wheat, and onion. In addition to onion, *F. proliferatum* is likely a troublesome pathogen on a variety of host plants including maize crops [8].

Online first accepted article

Table 1. Morphological characterization, isolation frequency, and relative density of *Fusarium* isolates in maize seeds

Isolate code	Name of fungi	Origin (tehsil)	Surface non sterilized seeds			Surface sterilized seeds		
			No. of Isolates	Fr	RD	No. of Isolates	Fr	RD
F1P	*Fusarium oxysporum*	Pasrur	15	4.5	30.61	5	2.5	22.72
F2P	*Fusarium solani*	Pasrur	7	1.5	28	2	0.5	28
F3P	*Fusarium verticillioide*	Pasrur	5	1.5	4.54	1	1	20
F4P	*Fusarium culmorum*	Pasrur	3	1.5	25	1	0.5	16.66
F1D	*Fusarium oxysporum*	Daska	10	3	20.40	4	1	18.18
F2D	*Fusarium solani*	Daska	4	1	16	1	0.5	14.28
F4D	*Fusarium culmorum*	Daska	2	1	16.66	1	0.5	16.66
F1Sa	*Fusarium oxysporum*	Sambrial	11	3.5	22.44	5	1	22.72
F3Sa	*Fusarium verticillioide*	Sambrial	5	1.5	4.54	1	1	20
F1B	*Fusarium oxysporum*	Badiana	9	3	18.36	3	2.5	13.63
F3B	*Fusarium verticillioide*	Badiana	3	1	27.27	1	0.5	20
F1Si	*Fusarium oxysporum*	Sialkot	4	1.5	8.14	1	0.5	4.54
F3Si	*Fusarium verticillioide*	Sialkot	3	1	27.27	0	0	0

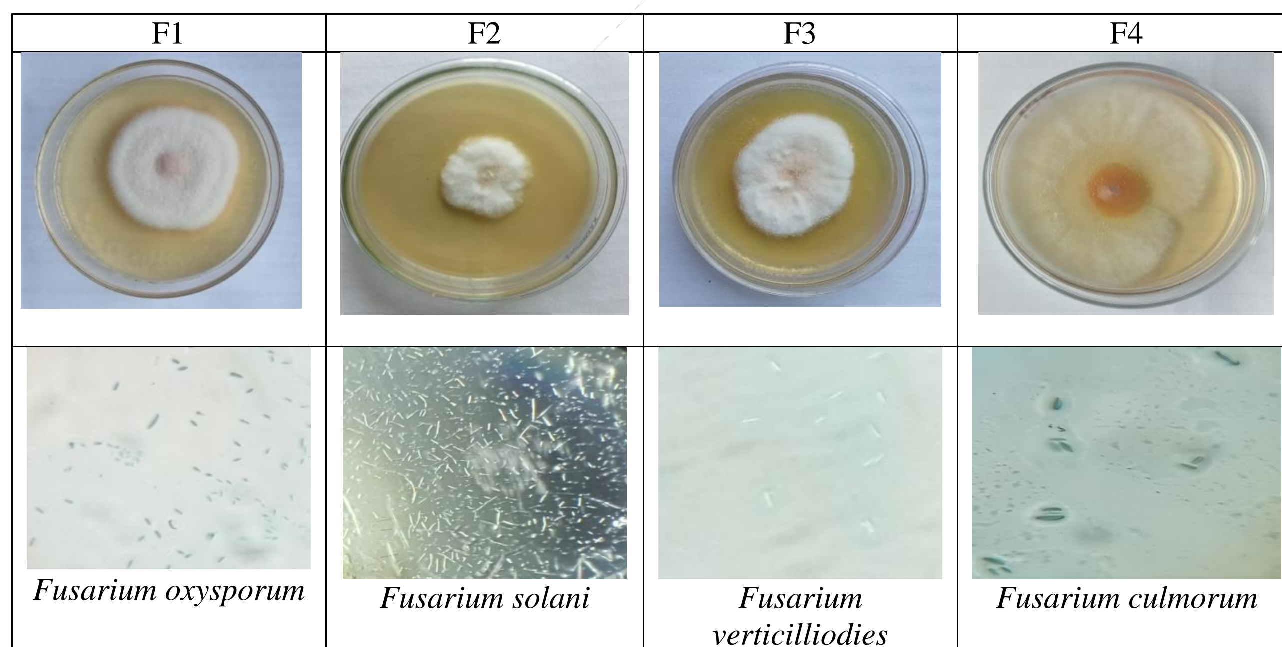

Figure 1. Colony and microscopic features of most common representative *Fusarium isolates* (F1, F2, F3, F4) isolated from maize seeds

Table 2. Pathogenicity Tests of *Fusarium* isolates on maize plants

Treatment	Total seedling	Wilted seedling	Disease incidence	Disease severity	Rating scale
F. oxysporum	10	6	60	60	3
F. solani	10	5	50	20	1
Control	10	0	0	0	0

Disease Incidence (DI) = No. of Infected Plants/Total No. of Plants×100
Disease Severity (DS) = No. of Infected Leaves/Total No. of Leaves in a plant×100

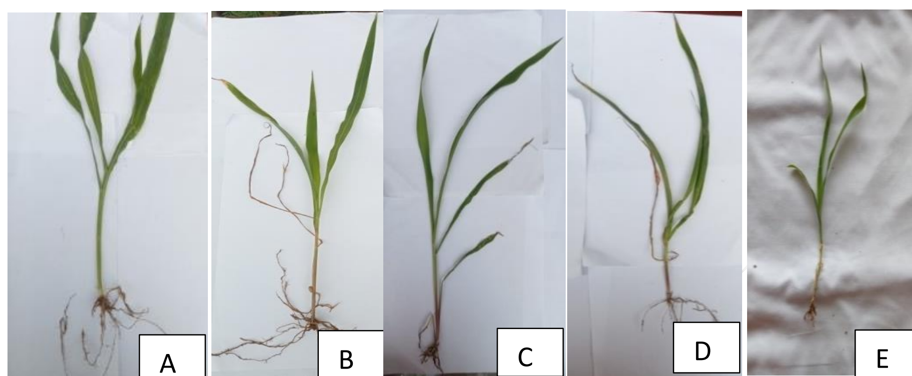

Figure 2. Thirty days post inoculation symptoms of four fusarium isolates on maize plants. (A) Control; (B) F1; (C) F2; (D) F3; (E) F4

Table 3. Effects of filtrated culture on the germination of seed and seedling growth

Treatment	Germination %	Root Length (cm)	Shoot Length (cm)	Vigor Index
F. oxysporum	10	0.5	0.0	5.0
F. solani	20	0.4	0.8	8.8
F. verticilliodies	20	0.2	0.1	4.1
F. culmorum	30	0.4	0.1	12.1
Control	70	0.9	1.5	64.5

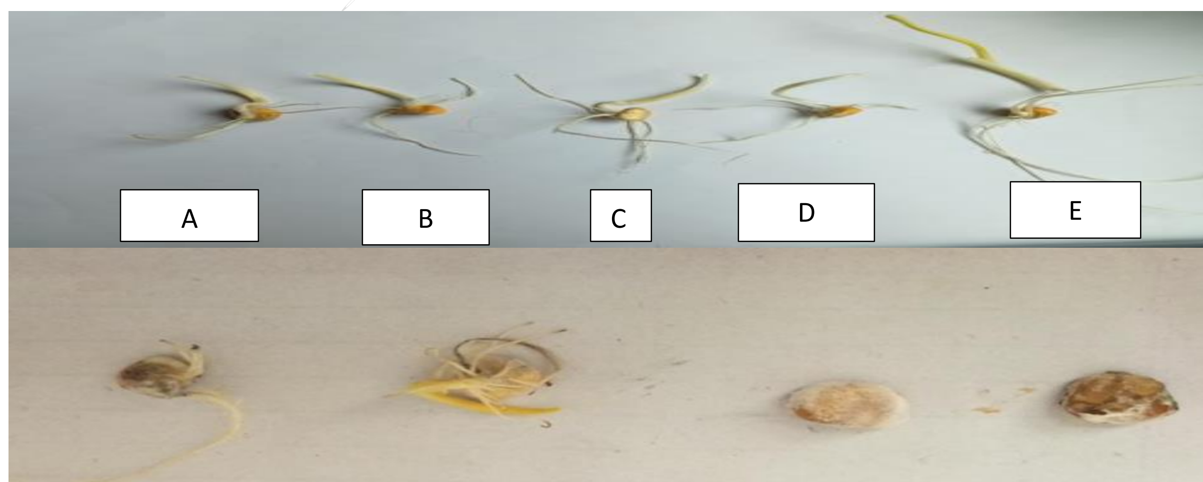

Figure 3. Seven days post-treatment effects of culture filtrates of *Fusarium* isolates on germination of maize seeds

Conclusion

Fusarium species are among the most familiar pathogenic communities of fungi that affect maize crops. In the present study, *Fusarium oxysporum* (F1) is the most frequent and pathogenic fungi among all the isolated species. The most suitable method for the isolation of *Fusarium* was the agar plate method which showed the highest isolation rate of *F. oxysporum* from both surface disinfected and non-disinfected seeds. Contamination of maize seeds with *F. oxysporum* might be the main cause of yield reduction in the Sialkot district. This baseline study would help to strengthen the crop protection efforts for the sustainable development of agriculture in the region.

Authors' contributions

Conceived and designed the experiments: A Batool & BG Nayyar, Performed the experiments: A Batool & A Naseer, Analyzed the data: A Sarwar & N Olikh, Contributed materials/ analysis/ tools: BG Nayyar & W Seerat, Wrote the paper: A Batool & A Sarwar.

References

1. Niaz I & Dawar S (2009). Detection of seed-borne mycoflora in maize (*Zea mays* L.). *Pak J Bot* 41(1): 443-451.
2. Tahir A & Habib N (2013). Forecasting of maize area and production in Pakistan. *J Crop Prod* 2(2): 44-48.
3. Ranum P, Pena Rosas JP & Garcia Casal MN (2014). Global maize production, utilization, and consumption. *Ann NY Acad. Sci* 1312(1): 105-112.
4. Tariq M & Iqbal H (2010). Maize in Pakistan–an overview. *J Agr Nat Resour* 44(5): 757-763.
5. Oerke EC (2006). Crop losses to pests. *J Agric Sci* 144(1): 31-43.
6. Van Asselt ED, Azambuja W, Moretti A, Kastelein P, De Rijk TC, Stratakou I & Van Der Fels Klerx HJ (2012). A Dutch field survey on fungal infection and mycotoxin concentrations in maize. *Food Addit Contam* 29(10): 1556-1565.
7. Walder F, Schlaeppi K, Wittwer R, Held AY, Vogelgsang S & van der Heijden MG (2017). Community profiling of Fusarium in combination with other plant-associated fungi in different crop species using SMRT sequencing. *Front Plant Sci* 8: 2019.
8. Logrieco A, Mule G, Moretti A & Bottalico A (2002). Toxigenic *Fusarium species* and mycotoxins associated with maize ear rot in Europe. Mycotoxins in Plant Disease: Under the aegis of COST Action 835 'Agriculturally Important Toxigenic Fungi 1998-2003', EU project (QLK 1-CT-1998-01380), and ISPP 'Fusarium Committee': 597-609.
9. Dean R, Van Kan JA, Pretorius ZA, Hammond Kosack KE, Di Pietro A, Spanu PD & Foster GD (2012). The Top 10 fungal pathogens in molecular plant pathology. *Mol Plant Pathol* 13(4): 414-430.
10. Lanubile A, Maschietto V, Borrelli VM, Stagnati L, Logrieco AF & Marocco A (2017). Molecular basis of resistance to Fusarium ear rot in maize. *Front Plant Sci* 8: 1774.
11. Scauflaire J, Mahieu O, Louvieaux J, Foucart G, Renard F & Munaut F (2011). Biodiversity of Fusarium species in ears and stalks of maize plants in Belgium. *Eur J Plant Pathol* 131: 59-66.
12. Sun G, Wang S, Hu X, Su J, Huang T, Yu J & Wang JS (2007). Fumonisin B1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. *Food Addit Contam* 24(2): 181-185.
13. Czembor E, Waskiewicz A, Piechota U, Puchta, M, Czembor JH & Stępien L (2019). Differences in ear rot resistance and Fusarium verticillioides-produced fumonisin contamination between Polish currently and historically used maize inbred lines. *Front Microbiol* 10: 449.

Online first accepted article

14. Oldenburg E, Hoppner F, Ellner F & Weinert J (2017). Fusarium diseases of maize are associated with mycotoxin contamination of agricultural products intended to be used for food and feed. *Mycotoxin Res* 33(3): 167-182.
15. International Seed Testing Association. (1999). International rules for seed testing. *Seed Sci & Technol* 24:1–35.
16. Garibaldi A, Gilardi G & Gullino ML (2004). Seed transmission of *Fusarium oxysporum* f. sp. lactucae. *Phytoparasitica* 32: 61-65.
17. Jaiswal S, Saini R, Sangani R, Tiwari S, Agrawal M & Agrawal MK (2012). Antibacterial activity of five fungal strains isolated from a leguminous soil field against nitrogen-fixing bacteria.
18. Jalander V & Gachande BD (2012). Effect of fungal metabolites of some rhizosphere soil fungi on seed germination and seedling growth of some pulses and cereals. *Sci Rep* 2(3): 265-267.
19. Panchal VH & Dhale DA (2011). Isolation of seed-borne fungi of sorghum (*Sorghum vulgare* pers.). *J Phytol* 3(12).
20. Leslie JF & Summerell BA (2006). Fusarium laboratory workshops-A recent history. *Mycotoxin Res* 22(2): 73-74.
21. Nayyar BG, Woodward S, Mur LA, Akram A, Arshad M, Naqvi SS & Akhund S (2018). Identification and pathogenicity of Fusarium species associated with sesame (*Sesamum indicum* L.) seeds from the Punjab, Pakistan. *Physiol Mol Plant Pathol* 102: 128-135.
22. Haapalainen M, Latvala S, Kuivainen E, Qiu Y, Segerstedt M & Hannukkala AO (2016). *Fusarium oxysporum*, F. *proliferatum,* and *F. redolens* associated with basal rot of onion in Finland. *Plant Pathol J* 65(8): 1310-1320.