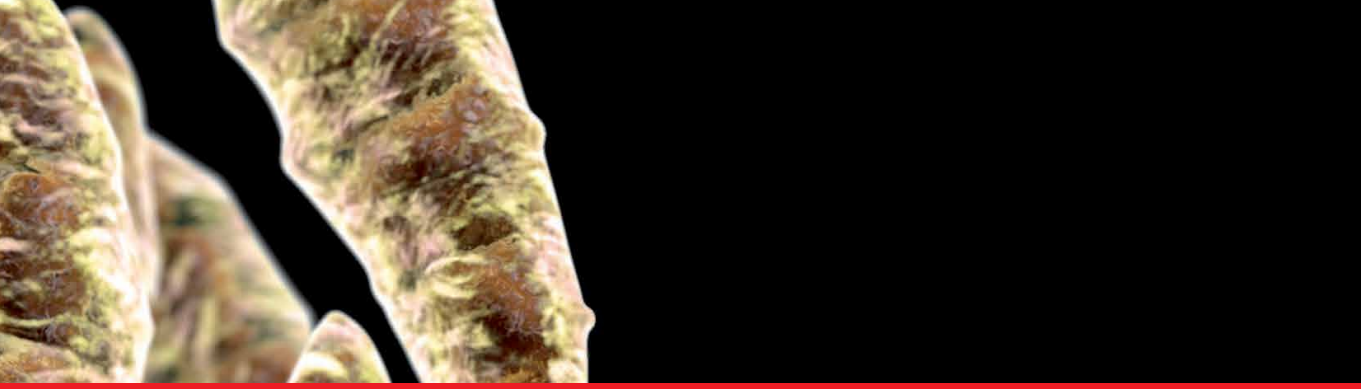

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Fusarium

An Overview of the Genus

Edited by Seyed Mahyar Mirmajlessi

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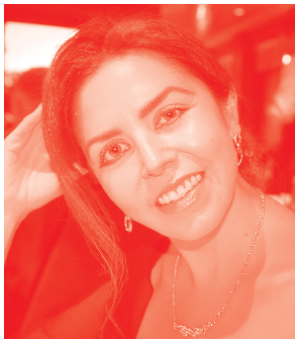

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Meet the editor

Dr. Seyed Mahyar Mirmajlessi is a highly experienced plant pathologist with expertise in disease management, plant-pathogen interactions, and biological control. He received an MSc in Plant Pathology with a specialization in the genetic diversity of plant-pathogenic fungi. He earned a Ph.D. in Molecular Plant Pathology from the Estonian University of Life Sciences. He continued as a postdoctoral researcher in plant protection at Ghent University (UGent), Belgium. Dr. Mirmajlessi has been contributing as a reviewer and editorial board member for a number of scientific journals. He has also published several research articles in various international peer-reviewed journals. Currently, he is a senior research associate at the Department of Plants and Crops, Faculty of Bioscience Engineering, UGent, Belgium.

Contents

Preface

Fusarium is a fungal genus belonging to phylum Ascomycota, subphylum Pezizomycotina, class Sordariomycetes, order Hypocreales, and family Nectriaceae. It is a large cosmopolitan genus of imperfect fungi with an estimated 1500 species. As plant pathogens, they cause root and stem rot, vascular wilt, and/or fruit rot in several economic crop species. *Fusarium* is one of the most important mycotoxigenic fungal genera in food and feed. However, they contain non-pathogenic strains that can colonize plant roots with an equal measure of pathogenic strains. The pathogenic strains are classified into host-specific forms (*formae speciales*) based on their host ranges, and further subdivided into pathogenic races according to their abilities to infect plant cultivars. Fumonisins, zearalenone, deoxynivalenol, and additional trichothecenes are well-known *Fusarium* mycotoxins. Though *Fusarium* research has improved our understanding of this group of fungi over the past decades, many traits of its biology still need to be addressed. Among *Fusarium* diseases, *Fusarium* wilts is a major problem all over the world. *Fusarium oxysporum, F. solani, F. fujikuroi*, and *F. graminearum* are the representative species known as plant-pathogenic *Fusarium*. Although *Fusarium* spp. use multiple infection strategies, these fungi are hemibiotrophs capable of transitioning from biotrophic to necrotrophic phases depending on environmental conditions. Since *Fusarium* is predominantly a soil-borne pathogen that survives for a long time because it is resistant to chlamydospores in soil, management of *Fusarium* diseases remains a challenge. This book provides an overview of recent research on *Fusarium* species in the fields of metabolites, pathogenicity, plant-pathogen interactions, and management strategies in agricultural practices.

I hope that this book will help a wide range of readers to update their knowledge of *Fusarium*. I would like to thank IntechOpen for inviting me to be the book editor. Special thanks go to Author Service Managers Jasna Bozic and Andrea Tomurad for their help and cooperation during the editing process.

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Section 1

Introduction to the Genus

Chapter 1

Fusarium Wilt: A Destructive Disease of Banana and Their Sustainable Management

Ram Niwas, Gireesh Chand and Ramesh Nath Gupta

Abstract

Banana is one of the most important fruit crops. The major losses in banana mainly due to the fungal wilt disease which is caused by *Fusarium oxysporum* f. sp. *cubense.* The pathogen is mainly soil bone and saprotrophic in nature that's why its management is very difficult. The yearly losses of banana by this disease in the world is ranging from 60 to 90% and in India 30–40%. Sustainable management of panama wilt is must to overcome these losses occur in banana. The management strategies for longer duration through crop rotation, organic amendment, application of micronutrient like silicon (Si), borax, host-pathogen interaction, hormonal induction of defence response, biological control, transgenic approach, disease resistance developed by somaclonal variation. These approaches are mainly emphasized for long term management of the panama wilt disease.

Keywords: *Fusarium oxysporum* f. sp. *cubense*, Fusarium wilt, banana, sustainable management

1. Introduction

Banana crops is as old as Indian culture and known to be one of the earliest fruit crops produced by humankind from ancient times in India with extraordinary socio-economic significance, interlink in the social and cultural legacy of the country. It is likewise the fourth most significant food crop after paddy, wheat and maize and forms an important crop for survival of farmers. Considering the wholesome importance of banana, it is so noticeable and well known among the Indians so that it is loved by both poor and rich individuals. It is otherwise called 'poor man's apple' since it is that the most economical among fruit grown up within the country with healthy qualities and wholesome values. The yearly losses of banana in the world are 60–90% [1] and in India 30–40% [2]. The most economically significant pathogen of banana is *Fusarium oxysporum* species. Fusarium wilt disease was earlier reported from Panama canal of Australia. In India, this disease was first reported by Stover [3] from West Bengal. The Fusarium wilt pathogen survives in soil and penetrates into the roots with the assistance of nematodes, from where it gradually spreads until it achieves the centre of the corm that is the reason the plant showed quick wilting. Purplish darker shading shows up in the xylem vessels and are blocked, external leaves turn yellow and finally breakdown. Soon, only a few

of the youngest leaves remain functional. Later the older leaves and pseudostem show yellow and longitudinal part with patches at the leaf edge. The symptoms become evident after 5–6 months of planting and are expressed both externally and internally.

2. Symptoms

The first internal symptoms develop in feeder roots at the primary sites of infection. They progress toward rhizome and are most noticeable where the stele joins the cortex. As the infection in pseudostem is colonized, blackout dark coloured streaks or flecks become apparent on and inside more seasoned leaf sheaths. Eventually, enormous segments of the xylem turn a black red to darker shading. The first external symptoms of Panama disease are a yellowing of the most seasoned leaves or a longitudinal splitting of the lower part of the external leaf sheaths on the pseudostem (**Figure 1**). This is further trailed by wilting and collapsing of leaves from the petiole base. At initial phases of infection, these leaves stay green. As the disease advances, more tender and young leaves breakdown until the whole plant covering comprises with dead leaves.

At the point when external symptoms are obvious on banana plants, however internal symptoms are missing from the pseudostem, it winds up for critically examine the rhizome. The plant sliced open at soil level to uncover the pseudostem base, and after that pushed over. Diseased plants have a trademark yellow to dim dark discolouration of the internal rhizome, which for the most part begins at the edges and advances inwards. Regularly some parts of the inward rhizome is influenced, yet with movement of the disease the whole internal rhizome winds up influenced. The external rhizome is rarely influenced. The piece of the rhizome that had been pushed over will show yellow strands of the rhizome which are appended

Figure 1.
External and internal symptoms of Panama wilt of banana.

to both the top and base portions of the rhizome. At the point when no discolouration is seen inside the rhizome, the outside symptoms are brought about by an option that is other than Foc. In such cases, the internal rhizome may show dark spots rather than the consistent yellow to reddish darker discolouration related with panama wilt.

3. Causal organism

Panama disease is brought about by the soilborne hyphomycete, *Fusarium oxysporum* Schlect. f. sp. *cubense*. It is one of more than 100 special forms of *F. oxysporum* that reason vascular wilt of flowering plants. It contains pathogenic and saprophytic strains that cannot be recognized morphologically. Fungus grow 4–7 mm/day on Potato Dextrose Agar medium at 24°C, with slight to significant fluffy mycelium, and have white to pink pigmentation. Sporodochia are tan to orange, and sclerotia are blue and submerged. Conidia and macroconidia are delivered on extended and unbranched monophialides. Microconidia are 5–16 × 2.4–3.5 μm, one or twocelled, oval to kidney shaped, and are borne in false heads. Macroconidia are 27–55 × 3.3–5.5 μm, four to eight sickle celled formed with foot shaped basal cells. Terminal and intercalary chlamydospores are 7–11 μm size, normally globose and formed independently or two by two in hyphae or conidia (**Figure 2**). Atypically for the species, chlamydospores are not distributed by isolates of *F. oxysporum* f. sp. *cubense* in vegetative compatibility group (VCG) 01214.

Four races of *F. oxysporum* f. sp. *cubense* have been revealed, just three of which affect banana (race 3 is a pathogen of Banana). Race 1 caused the pandemics on Gros Michel and furthermore affects the cultivars Maqueño, Silk, Pome, Pisang Awak. Race 2 affects cooking bananas *viz.*, Bluggoe, and some reared tetraploids. Race 4 is most dangerous since it affects race 1 and race 2 powerless clones just as the Cavendish cultivars. Up to this point, it had been accounted for just in subtropical regions where cold weather during winter are believed to be a predisposing factor. In any case, inside the most recent decade, remarkable harm was observed in Cavendish monocultures in tropical South-East Asia. A particular populace of the pathogen, VCG 01213-01216, is responsible for these flareups. Despite the fact that it is as of now limited to Asia and northern Australia, it caused critical disease in the western exchanges because of their dependence on the Cavendish clones. Vegetative or substantial similarity has been utilized widely to describe overall populaces of this pathogen. More than 20 VCGs have been accounted which is a pointer for the incredible genetic diversity that happens inside this taxon.

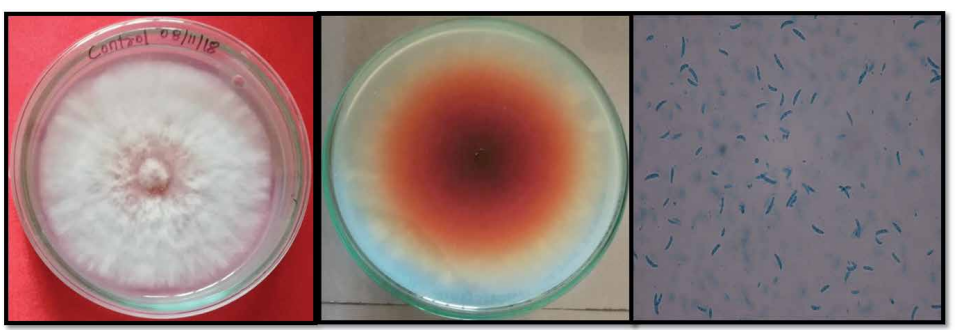

Figure 2.
Pure culture and spore of Fusarium oxysporium *f. sp.* cubense.

4. Disease cycle and epidemiology

Beckman and his colleagues considered the internal responses for susceptible banana cultivars to infection by *F. oxysporum* f. sp. *cubense*. They observed that race 1 strain of the pathogen produced rich microconidia in xylem vessels of cultivar Gros Michel. These propagules move acropetally in vessels by means of the plant transpirational change which caught at the scalariform parts of the bargains. As the fungus develops, it colonizes inside vessel end within 2–3 days, delivered microconidia on its adaxial side, along these lines empowering the pathogen to travel through another vessel (**Figure 3**). This procedure proceeded with unabated in Gros Michel, yet stopped in a race1 safe Cavendish cultivar not long after was it immunized. In the later case, gels formed in infected vessels in 24–48 h, followed by the development of vascular parenchyma into vessels after 48–96 h. These pathogen-incited exercises in the host caught spores of the pathogen and precluded it further colonization from claiming the host. At last, the host discharged phenolic aggravates that infused and lignified the blocking structures. Consequently, in a safe cultivar, there is a reasonable and quick coordination of host defences to ensure the systemic colonization of the xylem does not happen.

Rhizomes (suckers) are utilized generally as vegetative seed pieces for banana cultivation. Since they are generally free of symptoms when they are early infected by *F. oxysporum* f. sp. *cubense*, are a typical methods by which this pathogen is spread. The pathogen spreads in soil, running water, farm executes and apparatus. Work in the early trade estates showed that susceptible clones could not be successfully replanted in an infested site for as long as 30 years due to the long term survival of *F. oxysporum* f. sp. *cubense* in soil and as a parasite of nonhost weed species. Root tips are the characteristic, starting locales of infection; injured rhizome surfaces are evidently minor infection courts. Much of the time, root-tip infections are halted soon after the pathogen arrives at the xylem, and responsible for the formation of gels, tyloses and vascular breakdown. Macroconidia and chlamydospores

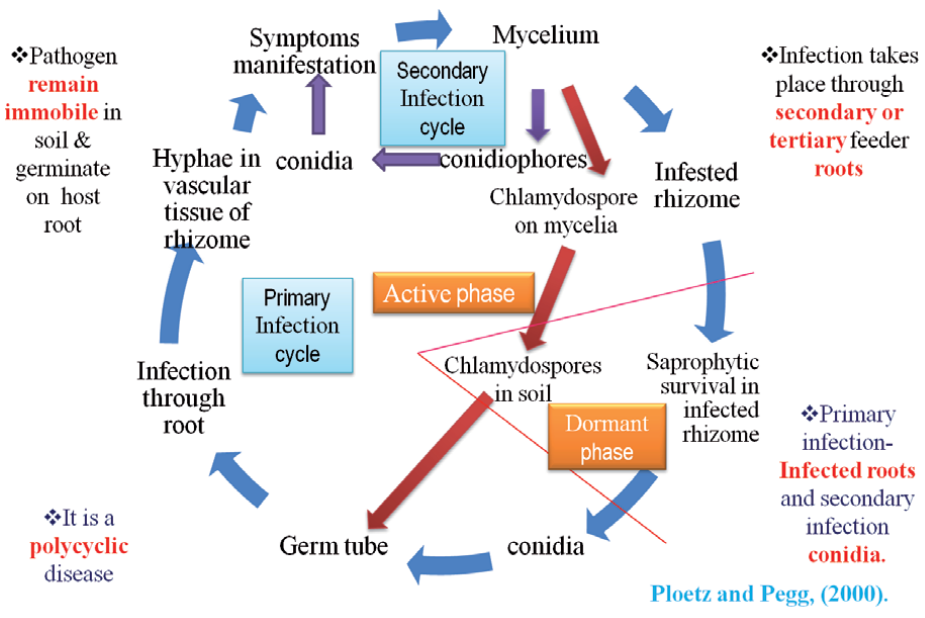

Figure 3.
Disease cycle of Fusarium oxysporium *f. sp.* cubense.

are formed on dead or passing on plants. The last propagules are the most significant survival structures of pathogen.

Weather factors like prolonged wet or dry conditions, extremes in temperatures, storm damage and soil conditions like poor soil drainage, aeration, unfavourable chemical or physical conditions also play a major role in influence on the wilt disease [4]. An internal water deficit due to dry conditions or waterlogging promotes expression of symptoms [5]. Temperature is a critical factor in panama wilt development [6]. Peng et al. [7] reported that the growth of Fusarium wilt pathogen is usually maximum at 28°C, and reduced above 33°C and below 17°C.

5. Management strategies for the Panama wilt disease of banana

5.1 Crop rotation

Continuous cultivation of bananas in the field compounds Foc event in the dirt. The spread and survival of this pathogen is mainly depend in the soil and also it can persist in the soil for long term, at the time of conducive environment causes of Fusarium wilt in bananas in severe form [5]. Yield turn as an administration practice, as a rule, is a profoundly effective and naturally friendly methods for control soil-borne diseases. In China, particularly in Panyu, Guangzhou, a region intensely infested by Foc, banana is turned with 2–3 years of economically developed Chinese leek (*Allium tuberosum*) to control Fusarium wilt [8]. Therefore, Chinese leek becomes a possible way for an ecologically friendly treatment to control Fusarium wilt of banana. Crop rotation is one of the most important cultural practices for reducing the plant pathogens in the soil. Crop rotation like pineapple-banana revolution was found more effective than maize-banana by reducing the Foc incidence in the banana fields.

5.2 Organic amendments

Organic matter management is basic for soil wellbeing and suppressiveness of the pathogens [9]. Albeit natural issues are included through yield buildups and spread harvests, offfield sources eq natural alterations (OAs) are especially significant as they are advanced with specific microorganisms. Yogev et al. [10] demonstrated that fertilizers dependent on plant squander buildups stifled diseases brought about by four different formae speciales of *F. oxysporum melonis*, *basilici*, *radicis lycopersici*, and *radicis cucumerinum*. Nonetheless, there are significant differences among banana and these yearly crops, regarding trimming cycle, yet in addition in the measure of auxiliary inoculum created per territory. An infected banana plant may created significantly more auxiliary inoculum than these yearly crops. Therefore, the degree of intercession to smother Foc inoculum with use of OAs may be more noteworthy and incorporated with other administration practices like utilization of beneficial and opponent microorganisms. In this sense, the helplessness of *F. oxysporum* to rivalry for supplements in the dirt may facilitate its concealment if great contenders are set up. For example, Fu et al. [11] revealed the concealment of FW in banana by the nonstop utilization of natural fertilizer. In any case, the effect of OAs on disease concealment may likewise be connected to natural control.

5.3 Application of silicon (Si) and borax (H_3BO_3): reduce the severity of Panama wilt of banana

Silicon (Si) helps in reducing the severity of a range of infections in specific crops [12]. In addition to the other strategies mentioned for managing banana panama wilt,

silicon (Si) application shows potential as part of a novel disease management strategy to avoid Foc infection and assist maintain enough banana output in the future [13]. It has been also reported that Si application suppressed disease in cucurbits caused by foliar and soil-borne pathogens. The obtained resistance of Si amended plants against the fungal pathogen might be due to accumulation of Si in the leaves, thereby, interfering with the pathogen's penetration as a result of a mechanical barrier. Niwas et al. [14] tested seven micronutrients *viz*., Calcium nitrate, Ammonium sulphate, Copper sulphate, Potassium chloride, Borax, Ferrous sulphate and Zinc sulphate. Borax @ 500 ppm completely inhibited the growth of *F. oxysporum* f. sp. *cubense* followed by zinc sulphate. Johnson et al. [15] also reported similar results on stem rot of groundnut. The micronutrients were used to manage the disease as well as it provided the healthiness of plant and increase the fertility of the soil that's why the plant was free from the disease or less infected. The mycelial growth of *F. oxysporum* f. sp. *cubense* was found low against different micronutrients and finally, it is concluded the borax completely inhibited the growth of *F. oxysporum* f. sp. *cubense in vitro* and used for the management of the Fusarium wilt disease of Banana under field conditions.

5.4 Application of phyto-hormones for the induction of resistance against Panama wilt

Plant hormones act as an important regulators in plant-microbe interactions. The impact of key plant hormones on the interaction between Fusarium wilt and host plants was also examined for suppressing the pathogens. Methyl jasmonate (MeJA) activates host defence against a wide range of infections as well as control host defence responses to biotic and abiotic challenges. Reglinski et al. [16] reported that application of MeJA to *Pinus radiata* seedlings resulted in induced resistance to subsequent wound inoculation with *Diplodia pinea*. Sun et al. [17] reported that the application of MeJA activated enzymes and reduced the level of H_2O_2 and malondialdehyde (MDA) in banana plantlets following inoculation with Foc TR4.

5.5 Application of bio-control agent for managing Fusarium wilt of banana

Considering the urgency of Panama disease, biological control offers a complementary disease management approach. However, there has been very little long-term biocontrol effectiveness studies for Fusarium wilt of banana in the field. In recent years, the usage of biocontrol agents (BCAs) has been shown to be an ecofriendly disease management technique. Xue et al. [18] identified one *Bacillus* spp. isolate as a possible biocontrol agent that plays a key role in the management of banana wilt disease. Despite the limitation of published scientific research on biocontrol, particularly with practical field findings, techniques that may be used to predict biocontrol failures in the field would necessitate a deeper knowledge of these interactions as well as pragmatic assessments of their usefulness. Biological control's success is determined not just by production techniques, but also by the expenses involved and the presence of effective antagonists. Furthermore, these antagonists must be dry preparations that may be stored for a long term. As field trials reports, soil application of *Trichoderma harzianum* effectively controlled Fusarium wilt with an efficacy comparable to that of the fungicide carbendazim. Previous reports have also demonstrated that siderophore producing endophytic *streptomycetes* from banana roots are effective against the Fusarium wilt pathogen and which developed as BCAs against the banana Fusarium disease. Successful inoculation of tissue cultured banana plants with fungal endophytes has been reported by Paparu et al. [19]. Addition of artificial inoculation to tissue cultured

banana plantlets resulted in a substantial reduction in the infection and severity of Fusarium wilt disease, as well as increased the plant growth parameters [20]. Application of plant growth promoting rhizobacteria (PGPR) to induce resistance against Fusarium wilt of banana plants. PGPR are considered as the most promising agent for cash crop production and managing soil-borne disease. Several substances produced by PGPR such as antibiotics have been related to pathogen control and indirect promotion of growth in many ways. Considering the employment of elicitors in crop protection is still in the very early stages for the use as a new control method, further research in this area is needed to demonstrate elicitors' effectiveness in banana wilt disease control.

5.6 Transgenic approaches for developing Fusarium wilt resistant banana

There are Fusarium wilt-resistant banana cultivars, and some have been conventionally developed through various breeding programmes. Now using techniques like particle bombardment or sonication assisted vacuum infiltration of the apical meristem, or using multiple bud clumps followed by Agrobacterium mediated gene transformation, genetic transformation of elite banana cultivars for resistance to Fusarium appears to be a more promising approach to improve disease resistance or tolerance. Testing the efficacy of the altered proteins against the target pathogen, which includes exposing the plants to the pathogen in an appropriate infective unit such as spores, is one of the most important phases in banana genetic engineering for disease resistance. Based on the antifungal activities of thaumatin like proteins are reported as a good candidate for genetic engineering toward production of disease resistant banana plants [21].

5.7 Breeding programme for the development of Fusarium wilt resistant banana

The most common, economical, successful and long-term Foc management method is universally recognized breeding and selection programme for disease tolerance or resistance [22]. Currently, Foc-TR4 resistance screening is mostly done on farmed bananas, but there is not much information on wild banana species. Li et al. [23] used a two step process comprising a combination of greenhouse and field studies which provided a comprehensive and reliable information regarding disease reaction on the evaluated genotypes of banana. To develop a global screening and evaluation protocol is critical for the selection of reliable resistant materials against the Fusarium wilt.

5.8 Somaclonal variations for development of Fusarium wilt resistant banana

Promising Foc resistant or tolerant clones acquired through nonconventional breeding techniques have been proposed as an aid in banana breeding programmes. Shoot tip cultures from banana clones are sensitive and resistant to Foc races 1 and 4 cultivated *in vitro* in the presence of fusaric acid and fungal crude filtrates to examine under *in vivo* and *in vitro* condition. Peroxidase activity was employed as a measure to distinguish between susceptibility and tolerance which was shown to correspond well with the host plant's field response to infections. At present, attempts to develop new banana genotypes resistant to Fusarium wilt using conventional breeding techniques face significant obstacles mainly because most cultivars of Musa AAA Cavendish subgroup are totally sterile and seedless. Whilst, several resistant clones has been also acquired through somaclonal variation. Wu et al. [24] investigated the utility of *in vitro* inoculation of rooted banana plantlets grown on modified medium as a reliable and rapid bioassay for resistance to Foc.

6. Conclusions

Fusarium wilt is a destructive disease worldwide. It is a soil borne and saprophytic in nature. Once a banana growing area is infected by Foc, it is very difficult for banana cultivation. Sustainable management requires proper know how of disease cycle and impact of weather factors for reducing the impact of Fusarium wilt. Combating the spread of Fusarium wilt is a race against time. To prevent and contain Fusarium wilt, a complementary approach such as exclusion and surveillance must be considered as significant components of integrated disease management strategies [25]. Thus, preventing the spread of Fusarium infection is very decisive for ensuring the continuity of banana production as well as securing the nutritional supply. Management strategies include crop rotation, organic amendments of soil, application of micronutrients like Si and Borax, plant-microbe interaction, induction of systemic resistance MeJA, treatment with bio-control agents like *Trichoderma harzianum* and PGPR to overcome the threat. Screening of banana genotypes for resistance to Fusarium wilt using in vitro evaluation, mutation and selection along with somaclonal variation which improve breeding efficiency for resistance against Foc. In addition to the these management strategies, various recent technologies introduced may shed some light into the development of Fusarium-resistant banana varieties which finally manage this venerable menace.

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Chapter 2

Potato Dry Rot Caused by *Fusarium* spp. and Mycotoxins Accumulation and Management

Huali Xue and Zhimin Yang

Abstract

Dry rot of potato (*Solanum tuberosum L.*) is an important postharvest disease during storage. The decay can be caused by several different species of *Fusarium* spp., such as, *F. sambucinum*, *F. coeruleum*, *F. oxysporum*, *F. avenaceum*, *F. culmorum*. The pathogen of *Fusarum* spp. causing dry rot of potato is considerable different in different countries and regions. The typical symptom of potato dry rot is sunken and wrinkled brown to black tissue patch on tuber with less dry matter and shriveled flesh. *Fusarium* spp. only invades host through wound or natural orifice during pre-harvest, storage and transportation period. Some *Fusarium* species infection associated with mycotoxins accumulation, which has phytotoxicity and mycotoxicoses in humans and animals. Synthetic fungicide is the main strategy to control the dry rot of potato, however, there are series of problem, such as environmental pollution, pathogen resistance. An integrated approach to manage the disease includes the introduction of resistant cultivar, appropriate cultural practices, and storage conditions combined with the application of synthetic fungicides pre-harvest or post-harvest. Moreover, some chemical fungicides and microbial antagonists have been integrated into potato dry rot management.

Keywords: *Fusarium* spp., potato dry rot, pathogenic mechanism, mycotoxins, control

1. Introduction

Fusarium is a large fungal genus within the Ascomycota phylum comprising a few hundred species that are mainly distributed in soil and in association with plants [1]. As we know, *Fusarium* spp. can cause dry rot of potato, it is a devastating pathogenic disease that significantly influences potato tubers (*Solanum tuberosum L.*) worldwide [2]. The disease not only causes a significant reduction in potato quality, but also leads to enormous economics losses. It is reported that 90% of potato tubers need to be stored for vegetable and industrial materials, however, the enormous yield losses attributed to dry rot disease during storage ranges from 6 to 25%, with up to 60% of tubers affected in some cases [3]. There are 13 species of *Fusarium* designated globally as causal agents of potato dry rot [4, 5]. Different species *Fusarium* were isolated from dry rot of potato tube and identified in different countries and regions. *F. sambucinum* is the most predominant pathogenic fungus causing potato dry rot in North America, China and some regions

***Fusarium* spp. species**	**Region**	**Source**
F. sambucinum	North Amercian, China and some regions of Europe	[3, 5, 6, 8, 10, 11, 13, 24]
F. coeruleum and	United Kingdom	[6, 14–19]
F. sambucinum		
F. sulphureum,	China, South Africa	[20–23]
F. sambucinem		
F. solani		
F. acuminatum		
F. avenaceum,	Finland and USA	[7, 25, 26]
F. equiseti,	North Dakota	
F. graminearum		
F. oxysporum	Egypt, Norway, Michigan	[27]
F. culmorum		[28]
F. verticillioides	Egypt	[29]
F. incarnatum		

Table 1.
Fusarium *species causing potato dry rot in different countries and regions.*

of Europe [3, 5–13]. *F. coeruleum* is the most prevalent agent associated with the dry rot of potato in cold storage in the United Kingdom and Great Britain [14–18]. Sometimes, *F. sambucinum* occasionally causes severe yield and economic losses in United Kingdom [6, 19]. In North Dakota, *F. graminearum* and *F. sambucinum* were reported to be the most frequent species *Fusarium* causing dry rot. In China, *F. sambucinum* is considered as the most notorious fungus in most potato growing regions [20], in addition, *F. oxysporum, F. avenaceum, F. acuminatum and F. equiseti, F. sulphureum*, *F. sambucinem* and *F. solani* also play the most predominant role in causing potato dry rot [21–23]. Similarly, *F. oxysporum* is the most common pathogen causing dry rot in Michigan. Recently in Egypt, the *F. sambucinum* was reported as the predominant fungus followed by *F. oxysporum*, *F. verticillioides* and *F. incarnatum* (**Table 1**) [29].

The frequency of the *Fusarium* species associated with dry rot is not only affected by crop location, but also by other factors such as potato cultivar, fungicide and seed tuber source [16]. In addition, *F. avenaceum*, *F. equiseti*, and *F. graminearum* are usually being considered lesser importance when compared with *F. sambucinum* and *F. coeruleum*; however, sometimes, they can be the predominant pathogen to cause serious disease in Finland and USA [24–26]. For example, A 2004–2005 survey of potatoes from stores in the north-central potato-producing region of the USA showed that *F. graminearum* along with *F. sambucinum* were the predominant causes of the disease [24].

2. Symptoms of dry rot, infection process of *Fusarium* and potato tuber tissue reaction

The symptom of potato dry rot includes sunken and wrinkled brown to black tissue patch on tuber with less dry matter and shriveled flesh. The initial symptoms of dry rot of potato appear on tubers at wound sites as shallow small brown lesions after

approximately one month of postharvest storage. The infected lesions enlarge in all directions, then the periderm sinks and collapses, eventually, the growing lesion may appear as concentric rings as the underlying dead tissue desiccates [1, 4]. Cavities underneath the rotted tissue are usually associated with cottony white, purple, pink or brick orange spore and mycelia of pathogenic fungus [30]. The whole rotted tubers always become shriveled and mummified (**Figure 1**). Dry rot lesions may be infected by some bacterial pathogens and cause soft rot decay, especially when the tubers are wet or stored at high relative humidity storage conditions [3]. *Fusarium* species that causes dry rot can also indicate themselves as seed tuber decay and in-field wilt.

Fusarium spp. cannot infect the healthy potato tuber through the stomata or lenticels when the tuber is in the absence of wounds. The pathogenic fungus successfully infect tuber only if the tuber's skin is ruptured [3], the fungus only invades the tuber through wound or natural orifice during pre-harvest or storage and transportation (**Figure 2**). The pathogenic hyphae are initial at intercellular, then become intracellular in dead cells. Histological studies showed that *F. coeruleum* infected through the intercellular spaces, the adjacent host cells remaining alive for some time; however, *F. avenaceum* infected through killing and penetrating the cells where it came into contact [3]. Lesions at the infected site can be prevented by the accumulation of suberin polyphenolic (SPP) and suberin polyaliphatic (SPA) [3, 31]. SPP and SPA can effectively prevent the spread of hyphae of the pathogen, and as wound periderm can be sealed with SPP and SPA [3]. In fact, the formation of SPP and SPA is the process of wound healing. Wound healing can suppress the development of dry rot by walling off infection sites and preventing lesions from expanding [3]. *F. sulphureum* infected tuber tissues were indicated to accumulate SPP and SPA [31].

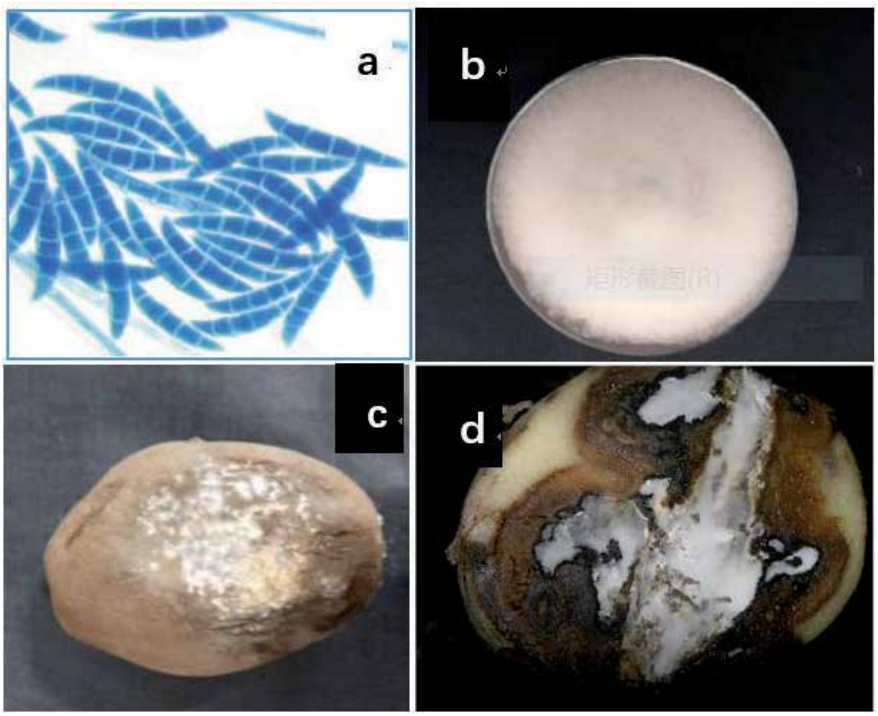

Figure 1.
(a) and (b) Respective spores and mycelia, (c) and (d) Respective typical symptoms of dry rot showing shriveled and mummified tubers.

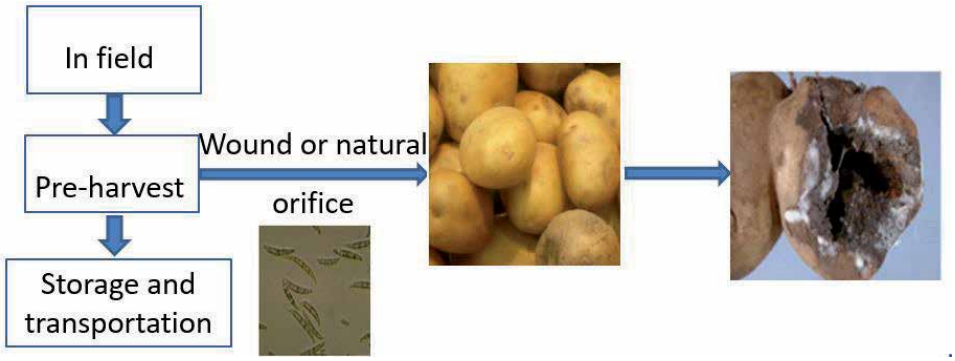

Figure 2.
Fusarium spp. infects potato tuber by wound or natural orifice.

The wound healing process includes two stages of wound-induced suberization: the closing layer formation and wound periderm development, accompanied by deposition of SPP and SPA on the wounded site [32]. It was reported that both SPP and SPA can resist bacteria and fungi invade by the formation of an effective physical barrier [33]. Jiang et al. [31] suggested some synthesis substances, such as benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid s-methyl ester (BTH) can accelerate the wound healing of potato tuber by elevation of phenylpropanoid metabolism.

3. Mycotoxins production associated with dry rot of potato

The dry rot, caused by some species of *Fusarium* spp., is associated with mycotoxins accumulation. Mycotoxins is a kind of secondary metabolites produced by pathogenic fungus under the favorable temperature and humidity condition, which can pose a potential risk to human health and food safety [34]. The mycotoxin produced by *Fusarium* spp. can be divided into two kinds of non-trichothecene and trichothecenes. The typical non-trichothecene produced by *Fusarium* spp. are listed in **Table 2**.

Beauvericin (BEA), enniatins (ENNs), zearalenones (ZEA), fumonisins (FUM), sambutoxin (SAM), fusaric acids (FA) and fusarin C (FUS) are usually detected in dry rot of potato tuber. BEA and ENN are cyclic hexadepsipeptides, which has antimicrobial, insecticidal, phytotoxic and cytotoxic characteristic properties [45]. ZEA belong to non-steroidal estrogenic mycotoxins, accompanied with estrogenic syndromes in some experimental animals [46]. FUM have been linked to leukoencephalomalacia, in horses and rabbits and have hepatotoxic and carcinogenic influences, as well as esophageal carcinoma in human, phytotoxic symptoms in plants [47]. SAM was detected in dry rot of potato caused by *F. sambucinum* and *F. oxysporum* [42, 44], which can lead to hemorrhage in the stomach and intestines, loss of body weight, feed refusal and death in rats. Consumption of FUS produced by *Fusarium* species has been associated epidemiologically with some diseases in human [46]. El-Hassan et al. [27] indicated that a significant positive correlation between FA accumulation and dry rot incidence.

The trichothecenes are the main kind of mycotoxins detected in dry rot of potato, which is a kind of chemically related sesquiterpenes compound. Presently, more than 190 known trichothecenes are detected. According to their chemical structure, they can be classified into four groups: types A, B, C, and D, the chemical structure are shown in **Figure 3**.

***Fusarium* species**	**Mycotoxins**	**Reference**
F. acuminatum	BEA	[35]
F. avenaceum	ENNs	[36]
F. crookwellense	ZEA, FUS	[37–39]
	FA	[40]
F. equiseti	FUM, ZEA	[27]
F. graminearum	ZEA	[37, 41]
F. oxysporum	FA	[40]
	SAM	[42, 43]
	FA, FUM, ZEA	[27]
	ENNs, BEA	[35]
F. sambucinum	SAM	[42–44]
	FA	[40]
	FA, FUM, ZEA	[27]
	ENN	[36]
	BEA	[35]

Note: BEA: beauvericin, ENNs: enniatins, FA: fusaric acid, FUM: fumonisin, FUS: fusarin C, SAM: sambutoxin, ZEA: zearalenones.

Table 2.
Non-trichothecenes produced by Fusarium *species in dry rot of potato.*

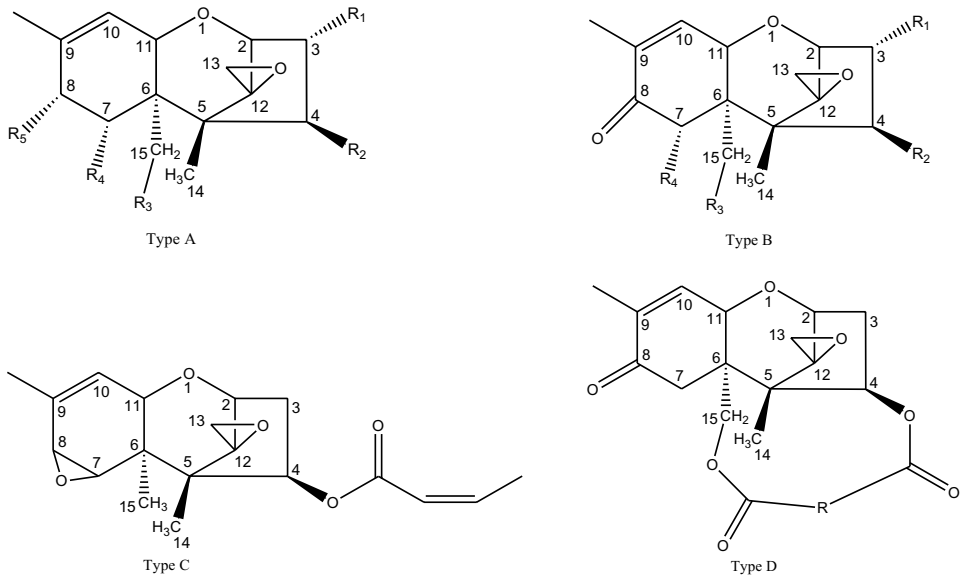

Figure 3.
Basic chemical structure of trichothecenes.

Types A and B are usually found in cereal grains, animal feed, and human food made from contaminated grains. In addition, they were also found in potato tubers infected by *Fusarium* spp. Trichothecenes have phytotoxicity and mycotoxicoses which can pose a severe threat for human and animal health [22]. The typical symptoms are vomiting, feed refusal, and diarrhea when animal intake the food

contaminated trichothecene, in severe case, trichothecenes have the potentiality leading to cancer, deformity and mutation [48]. Members of the genus *Fusarium* produce simple or non-macrocyclic trichothecenes while more complex macrocyclic trichothecenes are produced by fungi of the genera *Stachybotrys* and *Trichothecium* as well as other fungi [46]. Some *Fusarium* species associated with dry rot of potato were shown to produce trichothecenes in dry rot of potato tuber (**Table 3**). Type A and B are usually detected in rotted potato tuber tissue. Xue et al. [22] suggested that 3-ADON, T-2, FX and DAS were found in dry rot of potato caused by *F. sulphureum*, *F. solani* and *F. sambucinum*, Meanwhile, these mycotoxins were found not only in the lesion but also in the adjacent asymptomatic tissue, whose concentration showed a strong trend of decline with increase in distance from the infection point. Ellner et al. [55] indicated that 4,15-DAS and DAS were found in not only rotten tissue but also in distant healthy looking tissue in potato tuber infected with *F. sambucinum*, which had a strong decline in trichothecenes concentration with

***Fusarium* species**	**Mycotoxins**	**Sources**
F. coeruleum	DON, HT-2, 3-ADON	[49]
F. culmorum	DON, 3-ADON	[39]
	NIV, FX, DON, 3-ADON	[50]
F. crookwellense	NIV, FX	[37]
	NIV, DAS	[38]
	FX	[50]
F. equiseti	NIV, FX, 4,15-MAS, DAS, SCR	[50]
	T-2,	[27]
F. graminearum	DON, NIV, FX, 3-ADON, 15-ADON	[51]
	DON, NIV, FX	[37]
	NIV, T-2, 3,15-ADON, 15-SCRP	[52]
	NIV, FX, DON, 3-ADON, 15-ADON	[50]
	DON, 3-ADON, 15-ADON	[41]
F. oxysporum	T-2	[27]
F. sambucinum	DAS, MAS, NEO, T-2, HT-2	[53]
	DAS	[39]
	4,15-DAS, 15-MAS, 4-MASc	[54]
	DAS	[40]
	DON, NIV, HT-2	[49]
	T-2	[27]
	MAS, DAS	[56]
	3ADON, DAS, FX, T-2	[22]
F. sulphureum	3ADON, DAS, FX, T-2	[34]
F. solani	3ADON, DAS, FX, T-2	[34]

Note: 3-ADON: 3-acetyldeoxynivalenol, 15-ADON: 15-acetyldeoxynivalenol, DAS: diacetoxyscirpenol, 4,15-DAS: 4,15-diacetoxyscirpenol, DON: deoxynivalenol, FX: fusarenone X, HT-2: HT-2 toxin, MAS: monoacetoxyscirpenol, 4-MAS: 4-acetyl-monoacetoxyscirpenol, 15-MAS: 15-acetyl-monoacetoxyscirpenol, NIV: nivalenol, NEO: neosolaniol, SCR: scirpentriol, 15-SCRP: 15-acetylscripenol, and T-2: T-2 toxin.

Table 3.
Trichothecenes produced by Fusarium *species in dry rot of potato.*

an increasing distance from the visible rotted tissues. Similarly, Delgado et al. [51] reported that DON, NIV, FX, 3-ADON, 15-ADON were detected in dry rot of potato caused by *F. graminearum*, which also had the similar decline trend in trichothecenes concentration with an increasing distance from the rotted tissues.

In order to investigate the stability for heat, the effect of cooking on the trichothecenes was carried with potato tubers infected with *F. sambucinum,* the results indicated that the content of 4,15-DAS reduced 26% and 81% after 1 h and 4 h of cooking at 100°C, respectively [57]. The long cooking times required to degrade the structure of the trichothecens, which make it difficult that thermal treatment can be used as the degradation method for food or feed contaminated with trichothecenes. Although mycotoxins, considered thermally stable [57], are found in tuber invaded by different *Fusarium* spp., no sufficient data are currently available to evaluate the health risk for human health.

4. Dry rot control

4.1 Cultural practices and storage

The excellent cultural practices combined with appropriate of storage conditions are the most important and crucial factors which affect the incidence and severity of potato dry rot. In addition, planting healthy seed tubers in field, avoiding tuber injuries during harvesting, taking some steps to accelerate wound healing, providing appropriate storage conditions, these steps are the crucial factors, which provide good control to dry rot of potato [58]. In most cases, care is indispensable when harvesting that can minimize bruises and wounds for the harvested tubers. The tuber without wound may restrict the fungal spore colonization and germination, finally prevent major rotting. The 10–18°C temperature of the pulp is the suitable period for tuber harvesting [59]. The suitable temperature combined with high humidity (95–99%) and excellent ventilation is crucial for wound healing in tubers after harvest. After 7–14 days of vine killing, it is suitable for tuber to harvest, which has enough time to wound healing and reduce the chances of pathogen attack [59]. Planting certified seed tubers having <2% disease symptoms is recommended. The infected seed tuber is not recommended to introduce into field, because this will lead to pathogen survive during the whole growing period, finally cause dry rot. Moreover, proper disinfection treatment for storage facilities and implements used in handling and cutting of tubers are mandatory. Physiological maturation of the tuber is another important influence factor to affect dry rot development. Heltoft et al. [28] indicated that maturity plays an important role, in generally, late maturing cultivars are much more resistant to *F. sambucinum* than the early maturing one. The reason is maybe that the immature cultivar has high sucrose content and poor skin set, high sucrose can provide nutrition for pathogen growth, and poor skin are easy to bruise and produce wound, whose property make the early maturing cultivar are more vulnerable to pathogen [28]. Harvesting tubers with a high maturity can decrease the incidence rate of dry rot during storages. Harvest date is also an indispensable factor to influence *Fusarium* involved in dry rot incidence. Crop rotation is also very important, which is the most recommended cultural practice in managing soil-borne diseases in potato dry rot management [4]. Because *Fusarium* spores can live for a long term in soil and its broad host range, which makes it very difficult to manage by using crop rotation. Moreover, it is reported that crop infectivity of *Fusarium* isolates in clover and cereal crops that suggests that crop rotation may offer the favorable host and condition to survive for the pathogenic strains, rather than controlling them [13].

As we know, the dry rot of disease can infect through wound, when one single tuber is rotten, it can infect to other tubers around the rotten tuber, which will lead to a disastrous disease during storage. Therefore, it is necessary for any wounds (including pests and disease appearance) to have a thorough examination of tubers before storage, and that is the reason for proper grading before storage [60]. For storehouse, proper circulation of cool air is very crucial as respiration in stored potatoes generates excessive CO_2 and heat that can facilitate the growth of adhering fungal spores. The CO_2 concentration in a well-maintained storage facility is about 1200 to 1500 ppm. When the CO_2 concentration is more than 5000 ppm, which indicates storage rots and/or insufficient ventilation in the storage [60].

4.2 Host resistant

Host resistant play an important role in control postharvest disease. Xue et al. [34] compared two cultivars of Longshu No 3 (susceptible cultivar) and Longshu No 5 (resistant cultivar) susceptibility to dry rot disease and trichothecenes accumulation, the result showed that Longshu No 3 has more lesion diameter and the contents of FX, DAS, 3ADON and T-2 toxin in tubers inoculated *F. sulphureum*, when compared with the resistant cultivar of Longshu No. 5. In fact, resistance to each species of *Fusarium* is independent and genetically distinct [18]. The resistance to a species of *Fusarium* is transmitted to progeny, but appears to be associated with recessive alleles [61, 62]. Despite the fact that numerous potato cultivars and clones have been tested for susceptibility, no one cultivar is resistant to the whole *Fusarium* complex. Jiang et al. [31] suggested Qingshu 168 is resistant to *F. sulphureum*, and Longshu No 3 is susceptible to *F. sulphureum*. Clone B7200–33 from the USDA Potato Breeding Program appeared immune to both *F. sambucinum* and *F. coeruleum* [63]. Esfahani et al. [64] showed that the cultivar Saturna was relatively resistant to *F. sulphureum*, *F. solani*, and *F. oxysporum*. More recently, Yilma et al. indicated that the cultivar of Owyhee Russet was significantly higher resistance to dry rot than Russet Burbank. In general, some cultivars are susceptible to one species of *Fusarium* spp., but some cultivars are resistant to another species of *Fusarium*. Similarly, one strain of *Fusarium* is pathogenic to one cultivar, however, the strain of *Fusarium* is non-pathogenic to another cultivar. Because the susceptibility-resistance difference, on the one hand, was related with the strains and cultivars, on the other hand, the prevailing culture and environmental conditions in different regions of the world also play the important roles. Therefore, to optimize the cultivar to grow in field, it is essential to investigate the populations of *Fusarium* in the field and the pathogenicity.

4.3 Chemical control

The most popular and effective the management of dry rot is pre- and post-harvest management, that is to say, seed piece decay management before planting is combined with post-harvest treatments of tubers before storage. Presently, thiabendazole is the most effective and extensively used benzimidazole fungicide to control the dry rot disease caused by *Fusarium* species [1, 4]. Thiophanate-methyl (benzimidazole group) is widely used to manage seed tuber piece decay in Canada. However, the application of thiabendazole caused the appearance of resistant strains against *F. sambucinum*, but the rest of the *Fusarium* species *viz*. *F. solani*, *F. oxysporum, F. culmorum, F. equiseti*, *F. sporotrichioides, F. acuminatum* and *F. avenaceum* were still sensitive to thiabendazole [4]. Some new alternative“low-risk”fungicide, such as fludioxonil (phenylpyrroles) and azoxystrobin (strobilurins) also manifest good effect in managing dry rots [65]. Fludioxonil can be

used to control tuber seed piece decay and sprout rot. Daami-Remadi et al. [65] suggested that the synthetic fungicides such as, azoxystrobin, chlorothalonil and fludioxonil effectively reduced the severity of dry rot up to 50% when compared with control during 21 days of storage at 25–27°C. Fungicides mixture of metalaxyl + mancozeb or maneb is found effective in controlling tuber diseases where rotting was reduced by 50 and 91% on tubers inoculated with *F. sambucinum* and *P. erythroseptica* + *F. sambucinum*, respectively. Fludioxonil with mancozeb as seed tuber soaking treatment was effective to control dry rot [66].

The increasing resistance against fungicides, environmental pollution, and food safety problems, it is urgent to explore new and safe strategies to manage dry rot diseases. Some generally recognized as safe (GRAS) compounds, such as several inorganic and organic salts, essential oils and phytohormones, display good effect in sustainably managing dry rot of potato. Potassium metabisulfite and sodium metabisulfite showed 100% control of dry rot while magnesium sulfate, potassium sulfate, ammonium sulfate, sodium carbonate, sodium sulfate, calcium phosphate and potassium phosphites significantly reduced the infection percentage [67]. Li et al. [21] suggested sodium silicate significantly inhibited the growth of *F. sulphureum in vitro*, and suppressed the development of dry rot of tubers *in vivo*.

The use of chitosan as GRAS food additive is approved by the United States Food and Drug Administration. A recent study showed that a concentration of 0.25% chitosan completely inhibited *F. sambucinum* growth and prevented the other physiological losses in tubers. A similar antifungal effect of chitosan was reported against *F. sulphureum* and *F. solani* [68]. Chitosan and β- aminobutyric acid (BABA) applications suppressed the development of lesion diameter and trichothecenes accumulation in potato tuber inoculated with *F. sulphureum*, the involved mechanism is related with the increases in enzyme activities associated with induced resistance, and down-regulated genes involved in trichothecenes biosynthesis pathway [69]. Raigond et al. [70] also indicated that chitosan was effective in managing dry rots in a dose-dependent manner in potato cultivar Kufri Jyoti and Kufri Chipsona. As we know, chitosan can be as coating thin film material, in future chitosan should be used as a kind of coating thin film material to manage postharvest disease in potato tuber.

Recently, some other GRAS compounds, such as essential oils and plant extracts also manifested a good effect in inhibiting growth of fungus and the development of dry rot caused by *Fusarium* spp. *in vitro* and *in vivo* in the form of seed soaking or fumigation treatment [70, 71]. *Zanthoxylum bungeanum* essential oil found effective in reducing the severity of dry rot disease caused by *F. sulphureum* [72]. Similarly, the essential oils of fennel and peppermint also significantly inhibited the growth of *F. oxysporum* and controlled the tuber decay when applied as a protective emulsifiable concentrate [73]. The application of aqueous extracts of cinnamon markedly suppressed the growth of *F. sambucinum* and reduced dry rot [74]. The cinnamaldehyde, a predominant constituent of cinnamon essential oil, manifested excellent effect against *F. sambucinum*, the underlying mechanism revealed that a concentration of 3 and 4 mM inhibited spore germination by restricting the ergosterol biosynthesis, enhancing reactive oxygen species (ROS) accumulation and lead to disrupting cell membrane integrity. The down-regulation of ergosterol biosynthetic genes and the reduction of ergosterol content were analyzed by qRT-PCR and HPLC, respectively [75]. Some essential oils can directly inhibit mycotoxin accumulation, for instance, the essential oils of palmarose and clove inhibited DON and ZEA production in *F. graminearum*. The underlying mechanism maybe the essential oils down-regulate mycotoxin biosynthesis pathway. The essential oils and botanicals as sustainable alternative to synthetic chemicals needs to be further investigated for their on-farm efficacy in future.

4.4 Biological control

Numerous researches focused on the application of antagonistic microorganisms to the control postharvest diseases. Presently, antagonistic microorganisms are considered as an attractive alternative to replace synthetic chemical compounds to manage postharvest diseases. Bio-pesticide is considered more green and safety for the environment and human health than conventional synthetic pesticide. Antagonistic microorganisms can effectively control dry rot during the wound healing period when the potato tuber is at its most vulnerable. The first report that isolates from the genera *Pseudomonas Migula* spp., *Enterobacter Hormaeche* & *Edwards* spp., and *Pantoea Gavini*. spp. could decrease the incidence of dry rot caused by *F. sambucinum* [76]. Schisler's group also found two-strain mixtures of various antagonists manifested more effect than a single strain alone in controlling dry rot of potato [77]. *Pseudomonas fluorescens Migula* and *E. cloacae* were also effective under field conditions [78]. Glomus irregulare Blaszk., Wubet, Renker & Buscot enhanced defense responses, reduced the disease severity caused by *F. sambucinum* and modulated trichothecene mycotoxin gene expression in the pathogen [79].

Trichoderma is one of the most studied fungal genera, as well as recognized for its ability to inhibit different kinds of fungal pathogens and control both pre-harvest and post-harvest diseases. El-Kot [80] indicated that *Trichoderma harzianum Rifai*, *Epicoccum Link ex Steudel* sp., *Streptomyces endus Anderson & Gottlieb* had a high potential as biocontrol agent to manage dry rot disease caused by *F. sambucinum*. Daami-Remadi et al. [81] found *Trichoderma harzianum* and *Trichoderma viride* showed higher antagonistic activity against potato dry rot in Tunisia caused by *F. oxysporum*, *F. solani*, *F. graminearum*, and *F. sambucinum*. The major modes of action of *Trichoderma* is maybe that mycoparasitism, competition for nutrients, and the production of extracellular enzymes and/or secondary metabolites. Tian et al. [82] reported that antagonistic *Trichoderma* strains were able to detoxify DON, produced by *F. graminearum*, via glycosylation. Xue et al. [83] suggested that T-2 toxin (produced by *F. sulphureum*) at low concentration can be as an elicitor to induced resistance against dry rot of potato by stimulating reactive oxygen species (ROS) metabolism and phenylpropane metabolism. Yu et al. [84] reported that another biocontrol fungus, *Trichothecium roseum*, acting as an elicitor significantly enhanced defense responses in potato tubers against dry rot caused by *F. sulphureum*. During the defense responses, the resistance-related genes was up-regulated, the resistance-related enzymes and level of antifungal compounds significantly increased after treatment. Moreover, an arbuscular mycorrhizal fungus, *Glomus irregulare*, were found to modulate mycotoxin gene expression in *F. sambucinum*, inhibit its growth, and significantly reduce the production of DAS [85]. The application of mycoparasites as biocontrol agents that can manage plant diseases and detoxify/degrade mycotoxins is an ongoing topic of research [86].

5. Conclusion

Dry rot of potato, caused by *Fusarium* spp., is a disastrous disease postharvest during storage, which can cause economic losses and mycotoxin contamination. and the genetic diversity of *Fusarium* varies depending upon the geographical location and growing condition. The host cultivar and storage condition also significantly influence the frequency of occurrence and aggressiveness of potato dry rot. A breeding program is urgent to develop in order to adapt to the different cultivar against *Fusarium* species.

An integrated disease management strategy is necessary in order to efficiently management of dry rot, which includes appropriate harvesting conditions to avoid bruise for tubers, suitable storage conditions, as well as the pre- or postharvest application of registered synthetic chemical fungicides. In addition, the GRAS compounds and microbial antagonists as alternative strategies are being developed to control potato dry rot. The successful management of dry rot will certainly rest on additional research and development efforts between scientists and industry to implement an integrated strategy towards the efficient and durable management of dry rot.

In future, the application of omics technology will supply further functional genes and proteins that can be targeted for designing non-transgenic and transgenic management approaches. The integrated management of dry rot mainly depend on the additional research on the identified gaps and collaborative efforts of stakeholders (including researchers, industrialists and farmers) in developing an succesful management strategy from field to storage.

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Chapter 3

Fusarium Soilborne Pathogen

Leonce Dusengemungu

Abstract

Fusarium species are among the most persistent species of soilborne fungal pathogens. They cause severe economic damage in different agricultural production (potato, wheat, rice, etc.) due to the mycelia and chlamydospores that play a role during the infection of host plants. Our review has explored various studies on *Fusarium* species. The mechanisms involved in enhancing the protective ability of the *Fusarium* strain have been discussed. Furthermore, the current chemical and biological control methods to minimize Fusarium species' impact on crops were highlighted. Future directions in the attempt to improve the control of *Fusarium* soilborne pathogens have been discussed.

Keywords: fusarium, crops, soilborne, fungal pathogens, chemical methods

1. Introduction

Soilborne fungal pathogens are ubiquitous, and they can be found in soil, water, and air; when in contact with crops, they can trigger root rots, wilts, stunting, and other plant diseases [1]. The *Fusarium* species are classified among the most diverse soilborne pathogens [2]. Several research have pointed out that fungus from the genus Fusarium can grow on both live and dead plants and any other organic materials, including animal debris [3]. Furthermore, there is evidence that *Fusarium* conidia are waterborne and can transform into airborne when dehydrated or dried; their chlamydospores are predominantly soilborne [4]. The genetic structure of Fusarium and its sexual stages have allowed its ascospore to survive in extreme conditions like high temperature and high altitudes. Various *Fusarium* spp. have been isolated from humans and animals. In some instances, Fusarium species identified in the corneas of diseased eyes of humans have been linked with the loss of vision ability and more complications in immunocompromised personnel [5]. More findings have associated *Fusarium* spp. with different plant diseases such as head blight, vascular wilt in various crops, scab on cereal grains, and crown rot [5, 6].

Fusarium soilborne pathogens can resist harsh conditions and persist in soil due to the production of chlamydospores, which help them to survive without the host's support. Researches have shown that once the soil is colonized by *Fusarium oxysporum* f.sp.*cubense* (FOC), it is better to wait or use the plants that can resist *F.oxysporum*; otherwise, the susceptible varieties cannot survive [6, 7].

A biological method of soil disinfestation reported by (2012) was found efficient in controlling various soilborne pathogens, such as *F. redolens*, *F. Oxysporum f.spp. lycopersici*, *F. spinaciae*, *and radices-lycopersici*. The methods are accomplished by using labile carbon-activated microbial systems by creating anaerobic soil conditions in moist soils covered with polyethylene mulch. Furthermore, this reported method was also found effective in controlling some nematodes species such

as *Pratylenchus* and *Meloidogyne incognita* sp. [6]. Biological methods have been reported to ameliorate soil health by regulating the number of soil and plant pathogens due to their effect on agricultural residue accumulation [8].

2. The mechanisms involved in enhancing the protective ability of Fusarium strain

The biological management of Fusarium wilt diseases in soil and crops has been fulfilled by the use of nonpathogenic *Fusarium* spp. and other antagonistic organisms such as *Trichoderma* spp. (*Trichoderma harzianum*, *T. asperellum*, *and T. virens*) (**Figure 1**) [9]. The mechanisms involved in this process are still ill-defined. However, a few hypotheses involved in suppressing the occurrence of pathogenic Fusarium have been made through molecular mechanism elucidation and Fusarium species genome sequencing. Nutrient competition between pathogenic and nonpathogenic fungi has been noticed during the investigation of conducive and suppressive soils as well as population dynamics of soil supplemented with Fusarium spp., and it was revealed that the increase or decrease of Fusarium root colonization and chlamydospore germination were due to the nutrient competition [9, 10].

Competition of infection sites to the root surface was also described as a mode of action between pathogenic Fusarium and saprophytic fungi [11]. Larkin and Fravel investigated the effect of higher glucose concentration (0.2 mg/g of soil) on the germination of chlamydospores of nonpathogenic Fusarium (F047); it was noticed that the higher concentration of glucose suppressed the germ tube elongation of wilt Fusarium pathogen while inhibiting chlamydospore germination [12]. More research has correctly observed that nonpathogenic and pathogenic isolates

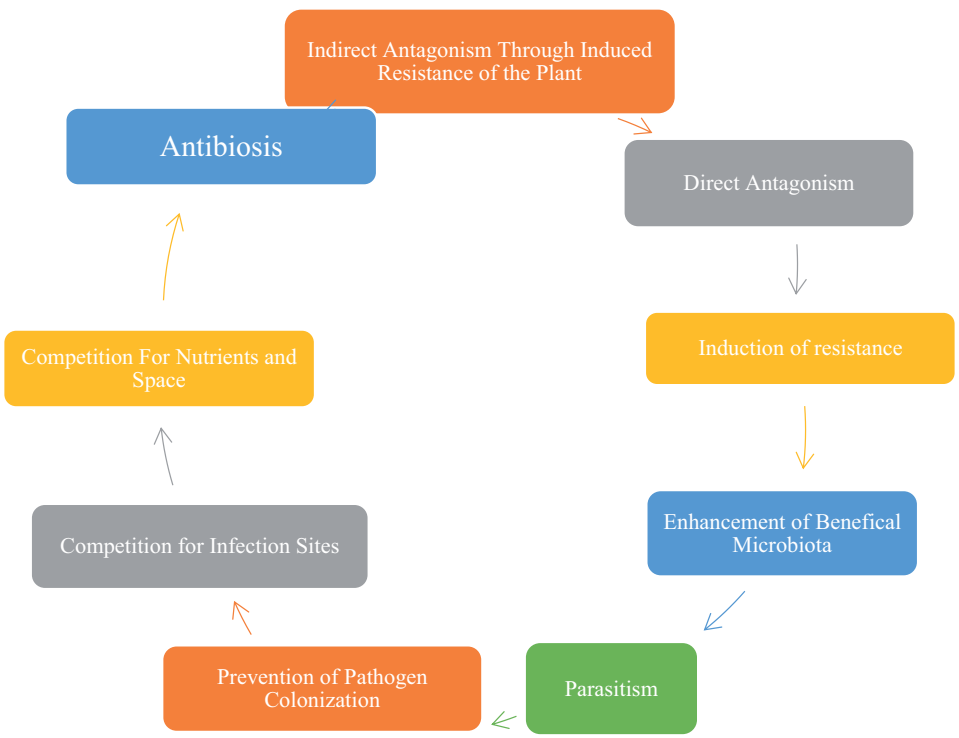

Figure 1.
The modes of action of the protective strains of F. oxysporum and many other beneficial microorganisms.

of Fusarium generally colonize root zones (emergency site of secondary roots, root apex, and elongation zone); these sites have higher nutrient oxidation [2, 10]. There is evidence that nonpathogenic *F. oxysporum*, which is characterized among endophytic fungi, can stimulate the defense response of host plants when plant pathogens attach them; furthermore, it has been found to increase resistance to environmental stress and enhance the production of essential hormones such as auxins and gibberellins, which are known to activate the plant growth [11, 13, 14].

The inoculation of nonpathogenic Fusarium strains into the roots of plants was found to inhibit the disease expression through a systemic resistance induction [15]. Nonpathogenic *F. oxysporum* were inoculated into watermelon plants to test their resistance against pathogenic Fusarium strains, and it was found to cause local and systemic resistance. In addition, the occurrence of both pathogenic and nonpathogenic strains on the root stimulated resistance mechanism in plants, therefore demonstrating their importance in the induction of local resistance [11].

3. Microbiological control of Fusarium soilborne pathogens

Current findings have shown that plant diseases resulting from soilborne plant pathogens' contamination are complicated to manage. However, various investigations have recognized the renowned biological control of soilborne pathogens using antagonistic microorganisms [16]. Previous studies have demonstrated that besides the most popular *Penicillium* spp., *Pseudomonas* spp., *Streptomyces* spp. (*Streptomyces griseoviridis*), and *Trichoderma* spp. (*T. harzianum*, *T. asperellum*, *T. koningii*), which represent the most broadly investigated groups of biological control agents, the Fusarium *species* can also be used to control plant diseases [2]. Among *Fusarium* species that cause soilborne pathogens, there is *F. emeriti*, *F. avenaceum*, *F. solani*, *F. sulphureum*, *F. tabacinum*, and *Fusarium oxysporum (F. oxysporum)*, which is also commonly known to cause vascular wilt in economically important crops. Among *F. oxysporum* include pathogenic and nonpathogenic strains. Research findings have pointed out that *F. oxysporum* as a biological agent can only control wilt originated from diverse pathogenic strains from similar species. However, more research is required to investigate if they cannot control wilt from other pathogenic species. Moreover, the mechanism involved in inducing the protective capacity of *F. oxysporum* is still not well understood [17].

Ortoneda et al. [18] investigated *Fusarium oxysporum* virulence mechanisms in plant and mammalian species. It was found that a single strain of *Fusarium* infection can induce vascular wilt disease in the plant. While the inoculation of microconidia of the tomato pathogenic isolates in the lateral tail vein of immunocompromised, mice can cause extensive complications such as the dissemination of infection in all organs and the death of the mice. More findings from the same study established that removing the mutant genes regulating a mitogen-induced protein kinase, a class V chitin synthase, and a pH response transcription factor affect diverse virulence factors important in both the tomato plants and mouse pathogenicity. Supportive studies have confirmed that *F. oxysporum* can suppress Fusarium wilts, and therefore, the utilization of this Fusarium strain to reduce the virulence capacity of other diseases due to Fusarium is recommended (**Figure 1**) [19, 20].

Relatively, little is understood about the interactions of plant pathogens, soil microbiome, and myxobacteria strains to reduce soilborne phytopathogens. Ye et al. [16] indicated that a predatory myxobacterium *Corallococcus* sp. strain EGB can be used to minimize cucumber Fusarium wilt through its capacity to colonize plant roots, thereby influencing the ability of the soil microbial community. The research findings done in two-year field experiment has shown that the inoculation

of the solid-state fermented *Corallococcus* sp. strain EGB controlled the cucumber Fusarium wilt by 79.6% in the greenhouses, 66.0% in the field in 2016, and 53.9% in the field in 2016, and the analysis of the capacity of strain EGB showed that it could improve the soil microbial community while reducing effectively the soilborne (*Fusarium oxysporum f.sp. cucumerinum*). Therefore, it was concluded that *Corallococcus* sp. has significant potential as a new biological control agent of soilborne pathogens, in particular Fusarium wilt. Due to the inefficient current techniques used to reduce vascular wilt pathogens in various important crops, more research is needed to explore and develop novel biological control agents and the currently available strains such as nonpathogenic *Fusarium*, *Pseudomonas*, *Streptomyces*, *Trichoderma*, *Gliocladium*, *and Coniothyrium* [21].

More research findings have confirmed that diverse bacterial and fungal strains can control Fusarium wilt in soil. A comparative analysis of meta-barcoding of taxonomic diversity of bacterial and fungal organisms from non-suppressive and suppressive soils concerning the control of Fusarium wilt has shown that bacterial and fungal strains recognized for their antagonistic activity against *F. oxysporum* was detected in suppressive and non-suppressive soils [22].

Fusarium wilt of banana (FWB), in particular, *Fusarium oxysporum* f.sp. cubense (Foc) race one has caused a considerable loss of banana plantations due to its distribution in tropical areas. However, researches show that FWB has been reduced up to 79% by employing *Pseudomonas* spp. and approximately up to 70% by various endophytes and *Trichoderma* spp. The use of another biological agent to control FWB is recommended to support the currently available techniques [22].

Actinomycetes obtained from soil have been found to inhibit Fusarium *Solani f.sp. pisi* that causes black root rot in Chickpea. A hundred actinomycetes were tested for their antifungal activities against *F. solani in vitro* and *in vivo*. The identifications result of actinomycetes used in the experiment showed that the isolates S3 of actinomycetes were highly similar to *Streptomyces* antibiotics, while the isolates s40 have similarities with *Streptomyces peruviensis*. From these results, it can be concluded that the actinomycetes and bacteria can minimize the effect of fungi. More studies should be conducted to produce these biocontrol en masse to confirm their biocontrol capacity and potential for commercialization as biocontrol agents [23].

4. Chemical control methods of Fusarium soilborne pathogens

Soilborne diseases can be reduced by spraying and fumigating with chemicals such as fungicides or biocontrol agents. Song et al. [24] investigated the capacity of seven fungicides, carboxin, azoxystrobin, hymexazol, tolclofos-methyl, thiram, carbendazim, and prochloraz, against *Fusarium oxysporum* Klotz on the Tomato (*Lycopersicon esculentum* Mill) plant grown in a hydroponic system. The inhibitory activities of these fungicides against the *F. oxysporum* findings showed that the median concentration (EC_{50}) was 154.03, 144.58, 69.961, 53.606, 26.292, 0.235, and 0.019 $\mu g.ml^{-1}$, respectively. Among all the fungicides used, prochloraz and carbendazim were found very efficient in controlling the mycelial growth of *F. oxysporum*. These results confirmed that wild tomato disease due to the infection of Fusarium pathogens could be inhibited by minimum toxicity of fungicides, using measured concentrations.

A similar study by Chauhan et al. [25] established the use of chemical fungicides, carboxin, carbendazim, quintozene, and thiram for seed management, pre- and post-sowing soil drench, and seed treatment of cotton can potentially reduce the occurrence of soil pathogens: *Fusarium oxysporum sp. Vasinfectum* (Atk.) Snyder and Hansen, *Macrophomina phaseolina* (Tassi) Goid = *Rhizoctonia bataticola*

(TAUB.) Butler, *Rhizoctonia solani* (Kuhn) and *Fusarium solani* (Mart.) Sacce. However, the use of the chemicals has several disadvantages, such as the inability to perform under various environmental and biotic conditions. Commonly used fungicides are usually inexpensive, but their efficacy is disputed due to the complications associated with diverse pest management strategies.

Nitrate nitrogen added to the soil at a higher pH has been used to control Fusarium wilt effectively [25]. A similar study has also reported that the use of nitrate-nitrogen significantly reduced the occurrence of Fusarium wilt on chrysanthemums, King asters, and carnations [26, 27]. Potassium quantity in soil has also been related to the occurrence of soilborne diseases and crop production. However, research has demonstrated that Fusarium soilborne pathogens incidence in tomatoes can be minimized by increasing potassium quantity in soil [28]. Similar studies have confirmed that high potassium levels can reduce the severity of Fusarium wilt in cotton [29]. The quantity of phosphate in soil has been investigating for its association with Fusarium diseases in crops. The findings revealed that higher phosphate quantity was associated with the occurrence of Fusarium wilt in muskmelon and cotton [30].

Numerous studies have established that the use of chemical disinfection to restore and prevent the occurrence of Fusarium wilt is not sustainable due to the environmental concerns because of the high toxicity and deteriorating effects of these chemical fungicides as well as the development of fungicides resistance; therefore, alternative control methods are recommended. Among the highly preferred methods include deep plowing, rotation, heating, grafting techniques, flooding, solarization, and various pesticides. Biofumigants and crop rotations are also among the environmental friendly methods that can be used to control soilborne pathogens especially Fusarium wilt. The methods to apply should be selected depending on the location and climate. Some methods such as soil solarization are ineffective where solar radiation is inefficient, while soil flooding requires a more extended period, approximately between 3 and 4 months, and is not preferred when the quantity of soil pathogens is high [6, 31–33].

5. Future directions

Plant-microbial interactions, ecological soil conditions, and the use of chemical and biological control agents to suppress soilborne pathogens play a significant role in the successful growth of plants. There is limited research investigating the importance of root exudates, intraspecific variation due to Fusarium infection. There is also a significant research gap in understanding the genetic control of Fusarium spore germination, its pathogenicity, and vascular occlusion that results in plant diseases. Therefore, these key research areas should be investigated further to ameliorate our understanding of the Fusarium organisms to improve the control of soilborne pathogens.

6. Conclusion

In our opinion, the combination of chemical fungicides and biological control agents can successfully inhibit soilborne pathogens, but more research is required to determine the effect of these methods on the soil microorganism's populations. The future success and effectiveness of these methods require rigorous testing of their protective ability and risk assessment. In addition, the influencing ecological characteristics of the soil should be determined accurately to enhance the effectiveness of these control methods. Moreover, more research is required to understand in detail the mechanisms involved in enhancing the protective ability of Fusarium

strain to enhance the industrial production of bio fungicides, safe formulation of chemical methods, and safe application procedures.

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Conflict of interest

The authors declare no conflict of interest.

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Section 2

Fusarium Wilt Management

Chapter 4

Current Status of *Fusarium* and Their Management Strategies

Amar Bahadur

Abstract

Fusarium spp. is one of the most economically important plant pathogens causing a wide range of plant diseases with significant crop losses globally. *Fusarium* wilt is a major problem all over the world. *Fusarium oxysporum*, *Fusarium solani*, *Fusarium fujikuroi* are economic importance species in worldwide. *Fusarium solani* causing disease in many agriculturally crops and favored by high temperatures and warm moist soils. The fungus produces three types of asexual spores; microconidia, macroconidia and chlamydospores serve as propagules in infecting host plants and found endophytes and saprophytes. The color of the colony, length and shape of the macroconidia, the number shape of microconidia and the presence or absence of chlamydospores are key features for the differentiation of *Fusarium* species. Pathogens, forms over 100 *formae speciales* cause disease in dicot and monocot plant species and infecting a variety of hosts. Vegetative compatibility Groups (VCG) is used to differentiate their races. Resistant cultivars and bio-control agents (*Trichoderma* spp., and *Psedomonas* spp.) have been used to manage the disease.

Keywords: *Fusarium* spp., *formae speciales*, symptoms, disease cycle, management

1. Introduction

Soil-borne pathogens caused infection in soil *via* the roots. *Fusarium* is a complex genus and worldwide distribution, causing diseases in plants, animals, and humans as well as the presence of non-pathogenic *Fusarium* in the natural ecosystem [1]. Fusarium wilt pathogen is one of the most destructive soil-borne pathogens around the world occurring in both saprophytic and pathogenic [2, 3]. Non-pathogenic and pathogenic *F. oxysporum* strains are in the soil, but the pathogenic strain causes severe vascular wilt disease in more than 150 agricultural crop species are banana, tomato, melon, watermelon, and cotton to be infected by vascular wilt [4]. Cereals and other food grains can be contaminated by Fusarium toxins and causes many diseases syndromes in mammals, moldy sweet potato toxicity, and poisoning in bean hulls [5]. *Fusarium* is one of the most important fungal genera that can produce mycotoxins. *Fusarium* mycotoxins are fumonisins, zearalenone, deoxynivalenol, and additional trichothecenes, cosmopolitan genus and numerous species are plant pathogens [6]. Cob rots in maize, caused mainly by *F. graminearum* and *F. verticillioides*, and both species produce mycotoxins which contaminate the grain and some strains of Fusarium solani cause collar rot of legume seedlings such as peas and bean. *Fusarium* species have their ability to grow on a wide range of substrates and their efficient mechanism for dispersal [7]. Many species are saprophytes which occur commonly in soil, colonize diseased roots, stems and grow quickly

on isolation media. It is important to test Fusarium isolates from diseased roots for pathogenicity to the plant. Diversity of host specificity within a single species into the 'forma specialis' each forma specialis exhibited a high level of virulence on a particular host species [8]. *Fusarium oxysporum* and their *formae speciales* that cause vascular wilt diseases, more than 100 Fusarium vascular wilt diseases worldwide and root rots cause with saprophytic strains as colonizing roots after the pathogen, forma specialis usually causes vascular wilt in only a single host specie. *F. oxysporum* mainly causes vascular wilt diseases, while *F. solani* mainly causes collar and root rots. Some strains of *F. oxysporum* can cause rots of melons and potato tubers. *Fusarium oxysporum* is an economically harmful species is a soil-borne phylogenetic diversified fungus with a wide host range including horticultural and grain crops that cause diseases such as wilt, rot, and damping-off [9, 10]. The pathogen has ranked fifth among the top 10 plant pathogenic fungi [11]. *Fusarium* wilt is one of the major diseases caused by *Fusarium oxysporum* strains and a major threat to agriculture [12]. Besides wilt disease, some strains can also cause foot- or root-rot resulting in serious yield losses in affected crops [13].

In the Cucurbitaceae family various *formae speciales* have been identified; among them, *F. oxysporum* f. sp. *cucumerium*, *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *melonis* are enormously important, whereas *F. oxysporum* f. sp. *niveum* is the most destructive pathogen of watermelon around the world [14]. Based on the host cultivar's resistance classified into four physiological races (0–3) [15]. The pathogen is responsible for yield losses of around 30–80% or even more [16, 17].

Fusarium is one of the most important groups of plant-pathogen causing diseases on crops. Plant-pathogenic fungi *Fusarium graminearum*, causes head blight of wheat and *Fusarium oxysporum*, which causes wilt and stem rot diseases [11]. *Fusarium* species produce a range of mycotoxins, most trichothecenes and fumonisins, which harm animal and human health [18]. *Fusarium* has a taxonomy with generic and species that diagnosing of diseases, identifying fungi, and developing management strategies. The generic concept of *Fusarium* was first diagnosed with the primary character of the banana-shaped conidia [19]. In *Die Fusarien*, which have a thousand species into 65 species, 55 varieties, and 22 forms in 16 sections and continued to use before 1960 [20]. In Russia, recognized and documented *Fusarium* [21, 22]. The Commonwealth Mycological Institute in the United Kingdom published The Genus *Fusarium* in understanding variability in *Fusarium* recognized 44 species and highlighted morphological characters, especially microconidia and the sexual reproductive structures, which used to differentiate species to identification [23]. A pictorial atlas to *Fusarium* that recognized more than 90 species [24]. A manual that recognized 41 species, and further 16 species were published [25]. *Fusarium* species use three predominant species concepts *viz*., morphological, biological, and phylogenetic to differentiate *Fusarium* species. Species concepts in *Fusarium* have been discussed [26]. Many "new" species of *Fusarium* discovered and described [27] and host species [28–30] are explored, grown in new areas [31–34]. Currently, there are more than 300 phylogenetically distinct species [35].

2. Pathogens (*Fusarium* spp.)

Fusarium species are widely distributed in soil, aerial plant parts, plant debris, and other organic substrates. A genus *Fusarium* is a large group of hyaline filamentous fungi [19]. Fusaria are common soil saprophytes and are also known as phytopathogens [36]. The genus Fusarium currently contains over 20 species [37]. Some Fusarium species have a teleomorphic state [38]. The commonest species include *Fusarium solani*, *F. oxysporum*, *F. equisetti* and *F. chlamydosporum* [39]. Two

Fusarium species were recently included in the list of the top ten plant pathogenic fungi with both economic and scientific importance [11]. This genus interacst with plants as endophytic root colonizers [40]. they may be responsible for a wide range of human infections [41]. *Fusarium* genus has more than 1500 species and several strains occur on plants/animals producing mycotoxins. *Fusarium* belongs to Phylum—Ascomycota, Order—Hypocreales and Family—Nectriaceae. *Fusarium* species are complex includes plant pathogens, human pathogens, and non-pathogens. Pathogenic strains are morphologically indistinguishable from nonpathogenic strains. *Fusarium* pathogens are persisting in the soil as chlamydospores, cause infect through the feeder rootlets and then colonize the vascular system, leading to severe wilting and death of plants. Important species are *Fusarium oxysporum*, *F. solani*, *F. fujikuroi* and *F. graminearum* well known plant-pathogens. It's characterized by fast-growing colonies with floccose aerial mycelium, colony pigmentation from pale, rose, burgundy to bluish violet depending on species and growth conditions. *Fusarium* usually produces pale violet to the dark magenta pigment in agar media (some do not produce). Conidia are often produced in sporodochia which are slimy dots in the culture, macroconidia are fusiform, multi-celled by transverse septa and characteristic foot-shaped basal cell pointed apical cell. Some species also produce microconidia are mostly single-celled, in some cases three to five celled and vary from globose, oval and fusiform. A few species produce microconidia in chains and others in slimy. *Fusarium* characteristics by morphological conidia in size and shape of macroconidia, the presence or absence of microconidia and chlamydospores, colony color and conidiophore structure [42]. Macroscopic and microscopic features, such as the color of the colony, length and shape of the macroconidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are key features for the differentiation of Fusarium species [43]. The *Fusarium oxysporum* is a soil borne fungi found in cultivated and uncultivated soils worldwide [7]. *F. oxysporum* have high functional and genetic diversity [6]. *F. oxysporum* can affect perennial and annual plants, including aquatic plants (lotus), cause wilts and crown rot on field crops, garden, ornamental crops and weeds (broomrape and witchweed). Strains with the same host range are grouped into *forma specialis*. In some *formae speciales* are subdivided into races by cultivar specialization [44]. Based on size and shape of macroconidia, presence or absence of microconidia and chlamydospores, colony color, and conidiophore Fusaria classified [42]. Morphological pictures of plant pathogenic, saprophytic and bio-control strains of *F. oxysporum* are indistinguishable. Based on taxonomic fusaria recognizing more than 100 species [23–25]. Pathogenic strain is very host specific, attacking only one or a few species and certain cultivars and designated as *formae speciales* and race of the pathogen. Proposed a system of classification of *F. oxysporum* strains, on basis of vegetative compatibility group (VCG), but not a universal tool of identify *formae speciales* or non-pathogenic isolates [45]. Nitrate reductase and phosphate permease have been used successfully to distinguish *Fusarium* species [46]. The presence or absence of microconidia is a primary character in *Fusarium* taxonomy. *Fusarium* teleomorphs have been described, classified into several different genera (*Gibberella*, *Nectria*) (**Figure 1**) [47].

2.1 Identification

There are three basic concepts for identification of *Fusarium* sp., by morphology can differentiate species, biological as sexual viable and phylogenetic as the common origin of the same species. Colonies character on potato dextrose agar of *Fusarium* species. *F. oxysporum* and *F. solani* can establish in suppressiveness soil than other species. *Fusarium* microconidia are oval to kidney-shaped, generally

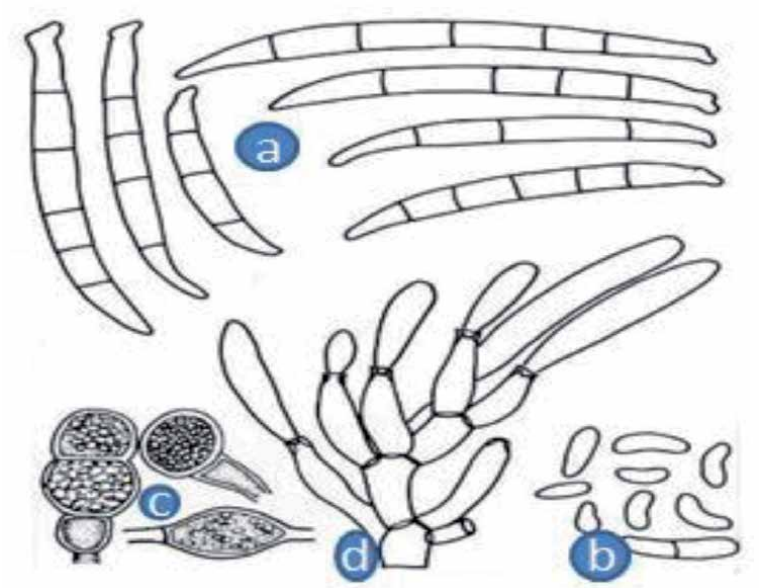

Figure 1.
(a) macroconidia, (b) microconidia, (c) chlamydospores, (d) conidia and conidiophores of Fusarium *spp.*

one-celled produce on short conidiophores on aerial mycelia and enter into the sap stream transported upward, macroconidia are fusiform having three to five cells and produced large numbers on sporodochia and chlamydospores are usually two types one within the macroconidium and other within the mycelium, formed singly/in pairs or chains with thick-walled, survive in the soil for a long time. A system of classification of strains of *F. oxysporum*, based on their vegetative compatibility as a described method based on pairing nitrate non-utilizing mutants to determine the vegetative compatibility group (VCG) of each strain and use of various molecular tools that group together genetically similarity in strains [45]. VCG cannot be used as a universal tool to identify *formae speciales* or nonpathogenic isolates only molecular tools can provide information for a taxonomic framework for species identification to relationships among species. Sequences of the β-tubulin region have been useful to distinguish some Fusaria [48]. Use nuclear restriction fragnment length polymorphism (RFLP) and VCG to determine *F. oxysporum* f. sp. *radicis-lycopersici* [49]. Use random amplified fragment length polymorphisms (RAPD) to differentiate races of *Fusarium oxysporum* f. sp. *vasinfectum* on cotton [50]. DNA sequences of the ITS regions are very useful in distinguishing species in many eukaryotic organisms, but not is very informative for Fusarium [51]. Random amplified polymorphic DNA identify sequence-characterized amplified region (SCAR) markers. Many *formae speciales* are known to be polyphyletic, making it difficult to identify specific molecular markers [52, 53]. Molecular methods, such as 28S rRNA gene sequencing, may be used for rapid identification of Fusarium strains to species and subspecies levels [54]. Polymerase chain reaction (PCR) based rDNA detection method [55] and detection of protein banding patterns by SDS-PAGE and esterase isozyme electrophoresis [56]. Cultures of Fusarium species grown on Sabouraud Dextrose Agar at 25°C produce wooly, cottony, flat or spreading colonies [57]. *F. oxysporum* are responsible for severe damage on many economically important plant species and show a high level of host specificity, based on infection of the plant species and plant cultivars they are classified into more than 120 *formae speciales* and races [58]. Molecular tools are providing species identification as well as evolutionary relationships among species.

2.2 *Fusarium oxysporum*

F. oxysporum is soil-borne pathogen that survive in the soil for a long time in the form of chlamydospores, penetrates the roots and colonizes in xylem vessels, systemic appear as yellowing, wilting, and death in plants. *F. oxysporum* are saprophytic and able to grow and survive for long periods on organic matter in soil and in the rhizosphere of plant species [59]. Some strains of *F. oxysporum* are pathogenic on plant species causing wilt and responsible for severe damage on many economically important crops and show host specificity based on the plant species and plant cultivars. They are classified more than 120 *formae speciales* and races [58]. Some strains can penetrate roots, but do not invade the vascular system [60]. *F. oxysporum* strains are responsible for two types of symptoms, such as vascular wilting and rotting. Vascular wilt resulting in yellowing and wilting of the plant [61]. Rotting of root without reaching the vascular system is called basal rot, stem rot, crown, root rot and also affect storage organs such as bulbs, corms, tubers and rhizomes. The first rot reported on lupine was caused by *F. oxysporum*

Sl No.	*Fusarium* species	Host crops
1	*F. oxysporum*	cereals, peas, beans, nuts, bananas, onions, potatoes, citrus fruits, apples, spices
2	*F. solani*	fruits and vegetables, spices
3	*F. avenaceum*	cereals, peaches, apples, pears, potatoes, peanuts, peas, asparagus, tomatoes in temperate climate
4	*F. cerealis*	cereals, potatoes
5	*F. culmorum*	Cereals, potatoes, apples, sugar beet in temperate climates
6	*F. equiseti*	cereals and fruits contaminated with soil, vegetables, nuts, spices
7	*F. graminearum*	Cereals and grasses in warmer to tropical regions
8	*F. poae*	Cereals, soybeans, sugar cane, rice from the temperate region.
9	*F. proliferatum*	Corn, rice, figs, fruits
10	*F. sambucinum*	cereals, potatoes
11	*F. semitectum*	nuts, bananas, citrus, potatoes, melons, tomatoes, spices
12	*F. sporotrichioides*	cereals, pome fruits
13	*F. subglutinans*	corn, pineapple, bananas, spices, sorghum
14	*F. tricinctum*	cereals from temperate regions.
15	*F. venenatum*	cereals, potatoes
16	*F. verticillioides*	corn, rice, sugarcane, bananas, asparagus, spices, cheese, garlic from warm to tropical regions.
17	*Fusarium sacchari*	sugarcane
18	*Fusarium moniliforme*	sugarcane
19	*Fusarium fujikuroi*	rice
20	*Fusarium mangniifera*	mango
22	*F. verticillioides*	maize
23	*F. pseudograminearum*	wheat and barley
24	*Fustiaria circinatum*	pinus

Table 1.
Fusarium species and their host causing diseases.

Sl No.	*'formae speciales'* (f. sp.)	race	host plants
1	*asparagi*	—	asparagus
2	*apii*	1 to 4	celery
3	*callistephi*	1, 2, 3	African marigold
4	*cubense*	1, 2, subtropical race 4, tropical race 4	banana
5	*cannabis*	—	hemp
6	*ciceris*	0, 1A, 1B/C, 2 to 6	chikpea
7	*cucumerinum*	1 to 3	cucumber, muskmelon, watermelon
8	*cepae*	—	onion
9	*conglutinans*	1 to 5	cabbage, radish
10	*carthami*	1 to 4	safflower
11	*chrysanthemi*	3 races	chrysanthemum, gerbera, daisy
12	*dianthi*	1, 2, 4 to 11	carnations
13	*elaeidis*	—	Oil palm
14	*fragariae*	—	strawberry
15	*gladioli*	1 and 2	gladiolus
16	*glycines*	—	soybean
17	*lactucae*	1 to 4	lettuce
18	*lagenariae*	—	bottle gourd, winter squash
19	*lupini*	1 to 3	lupine
20	*lentis*	1 to 8	lentil
21	*lycopersici*	1, 2 and 3	tomato
22	melongena	—	egg plant
23	*melonis*	1 to7	muskmelon
24	*niveum*	0 to 3	watermelon, squash
25	*pisi*	1, 2, 5, 6	peas
26	*phaseoli*	1 to 7 and 27	common bean
27	radicis-cucumerinum	—	cucumber, muskmelon, sponge gourd, watermelon, squash
28	radicis-lycopersici	—	Tomato, eggplant, *Cucurbitaceae* spp.
29	radicis-vanillae	—	vanilla
30	*raphani*	—	radish
31	*rapae*	—	*Brassica rapa*
32	*saragae*	—	witchweed
33	*saffrani*	—	saffron
34	*spinaciae*	1, 2	Spinach, beet
35	*tracheiphilum*	1 to 4	cowpea, soybean
36	*tulipae*	—	tulipe
37	*vasinfectum*	1, 2, 3, 4, 6, 8	cotton, okra alfalfa, soybean, tobacco
38	*zingiberi*	—	ginger

Table 2.
Important 'formae speciales (f.sp.)' and race of Fusarium oxysporum.

f. sp. *radicis-lupini*. The term "*radicis*" can differentiate rot-producing strains from wilt-producing strains. The "*radicis*" name of the *forma specialis* to allow for identification of the type of symptoms. Some *formae speciales* such as *cepae*, *lilii*, and *opuntarium*, cause rotting but are not referred as *formae speciales* "*radicis*-host plant name. *F. oxysporum* causes disease on vanilla, described to as *forma specialis radicis-vanillae*. Two different *formae speciales* are causing two types of symptoms in tomato as the *forma specialis lycopersici* causing wilt and *radicis-lycopersici* causing rot, **Table 1** [62–66].

2.2.1 Fusarium oxysporum *'formae speciales (f.sp.)' and race*

Host range of plant species are grouped into a *forma specialis* and subdivided into races by cultivar specialization [44]. More than 100 *formae speciales* of *F. oxysporum* causing diseases in different plant species. *Forma specialis* is determined by testing the fungus for pathogenicity on various plants species and race is determined by pathogenicity on cultivars of a single plant species. Molecular tools can identify pathogenic strains and in some cases races of the pathogen. A *forma specialis* of fusarium fungus normally affects only one primary host species, but colonize endophytically in the roots of secondary hosts. Many *formae speciales* were named according to the host plant either the genus name/species name. A reported 106 *formae speciales* and 58 additional host plants which have no *forma specialis* as characterized and races based on cultivar identified 25 of the 106 *formae speciales* (**Table 2**) [67].

3. Diseases

The genus Fusarium species cause vascular wilts, root, stalk and cob rots, collar rot of seedlings, and rots of tubers, bulbs and corms, some species also produce mycotoxins in contaminating grain, diseases as ear and kernel rot of corn, scab of rice and wheat and stalk rot and grain mold infection of sorghum. *Fusarium* species are causing diseases such as crown rot, head blight and scab on cereal grains; vascular wilts on a wide range of horticultural crops; root rots; cankers; and other diseases such as pokkah-boeng on sugarcane and bakanae disease of rice. Wilts are important in many cultivated crops. *Fusarium* pathogens survive as chlamydospores in soil for long periods. Wilt pathogens colonize in the root cortex of some non-host plants. Chlamydospores form in the cortex when the plant dies. *Fusarium* produces harmful secondary metabolites known as mycotoxins [18]; toxicity to animals, humans, plant pathogens, and also in food and feeds [68]. Mycotoxins are secondary metabolites produced by *Fusarium* species and threat to animal and human health. Earlier infections during the harvesting some of them produce mycotoxins in agricultural products [69, 70]. Mycotoxins, are the trichothecenes, fumonisins, and zearalenone [71]. Vascular wilt fungus produces the characteristic xylem vessel clogging and wilting of plants. Colonization and clogging of vessels in addition to the secretion of several toxins by the fungus including fusaric acid, lycomarasmin, dehydrofusaric acid, play a major role in the development and progression of wilt symptoms [18]. First detected fusaric acid (in-vivo) in wilted cotton plants and suggested that responsible for the production of wilt symptoms [72]. Fusaric acid is a toxin in tomatoes and cotton [73]. Fusaric acid is well-known for its phytotoxicity and role in the pathogenesis of Fusarium wilts [74]. Fusarium species as plant pathogens, causing diseases such as crown rot, head blight, and scab on cereal grains; vascular wilts on a wide range of horticultural crops root rots; cankers; and other diseases such as pokkah-boeng on sugarcane and bakanae disease of rice [23].

4. Symptoms

The pathogen colonizes in the xylem, growing up the vascular system in the stem, disease development and symptom expression of host plants depend on the colonization of vessels by the pathogen [75]. Hyphae and chlamydospores of diseased plant debris in the soil infect young rootlets and enter the xylem vessels. Colonization in the plant causes a reaction, producing brown phenolic compounds and tyloses. Browning of vascular tissue is a key symptom of pathogens that cause vascular wilt disease. Blocking of the xylem decreases water movement, causing the infected plant to wilt and die. Yellowing, wilting and stunting are general symptoms of many diseases of the root and stems. Early symptoms appear as leaf yellowing, slight wilting during the day and stunting. Wilt starts vein clearing on the younger leaves and drooping of the older lower leaves, followed by stunting, yellowing of the lower leaves, defoliation, marginal necrosis and plant death. This seed and soil-borne plant pathogen showing symptoms like chlorosis, necrosis, immature leaf fall, vascular system browning, and finally wilting. *Fusarium* vascular wilt diseases are more severe in warm, wet conditions. The *Fusarium* infects through wound sites as made by the nematode as associated with roots. Pathogenic strains of *F. oxysporum* are produce two types of symptoms, vascular wilting and rotting, penetrates the host roots and reach the xylem vessels, colonizes caused vascular wilt, and progressive yellowing and wilting [61].

4.1 Disease cycle

Fusarium is a monocyclic, soil-borne, diversified fungus including pathogenic and saprophytic [9, 10]. Dispersing by soil, plant debris, farm machinery [76] and seeds [77] and survive more than 15 years without host plants [78]. Pathogens spread through water and farm equipment over short distances but extensive areas through contaminated soil, seeds, or seedlings. A report indicated that spread by seeds [79]. Fusarium wilt does not spread from plant to plant within a season. The fungus infects the plants by germinating spores, growing through the wounds and openings near the root hair [80]. Fungal hyphae penetrate the vascular tissue produce microconidia [80]. Microconidia are released into the xylem, which travels upward though the water and colonizes the vascular tissue [81]. In stressful environmental fungi produce chlamydospores into the soil. *Fusarium* wilt accomplishes by spreading chlamydospores as the primary survival of the pathogen [15].

5. Management

Several *Bacillus* spp. strains suppressive effect against plant diseases caused by soil-borne diseases. *B. subtilis*, produce volatile compounds and activate plant defense mechanisms by triggering induced systemic resistance [82, 83]. Bacillus-mediated plant growth promotion due to promote phytohormone, biosynthesis, thereby enhancing nutrient uptake ability in the host and stimulating plant defense responses against biotic and abiotic stresses [84, 85]. *Bacillus* species can produce lytic enzymes like chitinase and β-1,3-glucanase, involved in the degradation of the fungal cell wall [86]. *Trichoderma* spp. show a wide genetic diversity, and are producers of several extracellular proteins, enzymes. Arbuscular mycorrhizal fungi (AMF) protect plants against phytopathogens and abiotic stresses [87–89]. Chemicals can prevent infection, but do not cure a plant once infected and these compounds affect beneficial soil microbiota and accumulate in the food [90]. Resistant plant varieties carrying resistance genes is currently the most effective

in terms of economy, ecology, and disease control. However, genetically encoded resistance is seldom durable and sooner or later new races emerge that overcome resistance [91, 92]. *Fusarium oxysporum* resistance genes are not available in the germplasm of all crops [93].

F. oxysporum are genetically varied in phytopathogens, saprophytes and bio-control agents. Management of *Fusarium* wilt use broad-spectrum chemical fumigates in the soil before planting that are environmentally unsafe and also living thinks, only cost-effective, environmentally safe method is resistant cultivars when these are available. Resistant crop varieties are available against some *Fusarium* wilt pathogens. However, resistant variety is not resistant to all races of the particular forma specialis. In case develop new races of the pathogen overcome host resistance. Managing Fusarium wilt is very difficult to manage because of chlamydospores persistent in nature for about 10 to 15 years and the development of new physiological races [15, 94]. *Fusarium* wilts diseases are difficult to control because the chlamydospores persist for a long time in soil. These fungi can survive infecting the root cortex of some symptomless, non-host crops. Only biocontrol agents are useful in the management of diseases. Non-pathogenic strains generally developed as bio-control agents and show several modes of action in their bio-control capacity, easy to mass-produce and formulate. The use of nonpathogenic strains of F. oxysporum to control Fusarium wilt has been reported for many crops [94–104]. On the infection sites on the roots trigger plant defense reactions; plants protect themselves from microbes by activating defense reactions such as systemic acquired resistance. During growth, plants are continuously challenged by a wide spectrum of environmental stimuli, by abiotic and biotic. Plants usually protect themselves from microbes by activating defense reactions such as systemic acquired resistance (SAR) after recognizing microbial stimuli. When plants are exposed to abiotic stimuli, the plants can acquire an improved defense by chance. Chemical stimuli, such as probenazole (PBZ), acibenzolar-S-methyl (ASM), tiadinil (TDL), and isotianil, have been used as plant activators and can induce disease resistance in plants. Foliar spray with validamycin A effectively controls soil-borne *Fusarium* diseases tomato wilt, and banana panama disease by inducing SAR. Once soil-borne fusaria pathogens spread in the field, their removal is very difficult. Soil treatments often are less sufficient and need to reduce their usage because of adverse effects on the environment. Biological control and resistance cutovers are alternatives to control fusarium diseases. *Trichoderma lignorum* was registered as a fungicide on the Agricultural Chemicals Regulation Law in Japan in 1954 to control *Rhizoctonia* disease in tobacco. This was the first registered bio-fungicide in the world. A non-pathogenic strain of F. oxysporum was registered in 2002 as a bio-fungicide to control soil-borne wilt of sweet potato plants caused by *F. oxysporum* f. sp. *batatas*. *Trichoderma atroviride* was registered as a bio-fungicide to control rice 'Bakanae' by seed or nursery-box treatment.

6. Conclusion

Fusarium is a large genus of imperfect fungi and numerous species are important plant pathogens. *Fusarium oxysporum* all strains are saprophytic, based on phenotypic and genetic characterize the strains and showed the diversity. Interactions between pathogenic and non-pathogenic strains result in the control of the disease. Complex fusarium species are the economic importance of their pathogenic/non-pathogenic activity. The development of molecular-based genomic tools to study in relation and its characterization. As 106 *formae speciales* have been clearly described within *F. oxysporum*. The pathogenic activity of *F. oxysporum* on plants of economic interest, many wild plants also infect by new *formae speciales*. Greater diversity in

F. oxysporum and within *formae speciales* may be revealed over time by using new plant genotypes resulting from breeding. Fusarium species has a significant role in socio-economic and international trade for food security as ability to destroy crop yields and contaminate plant products. New populations of *Fusarium* pathogens will continue to emerge through micro-evolution and the invention of exotic pathogens. Need the research on the biology of the fungus to determine their role of non-host crops and length of survival of chlamydospores in soil.

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Chapter 5

Fusarium Disease of Maize and Its Management through Sustainable Approach

Zerald Tiru, Parimal Mandal, Arka Pratim Chakraborty, Ayon Pal and Sanjoy Sadhukhan

Abstract

Fusarium causing disease in maize is probably the one of the most serious diseases among the crop plants all over the world. It not only damages the maize plant, reduces its potential yield and its nutritional values but imposes threatening to the human life through the induction of mycotoxin development. *F. graminearum* and *F. moniliforme* syn. *Fusarium verticillioides* are two important maize pathogens that cause substantial damage to its ear, stalk and foliage, causing contamination of grains with mycotoxins. Since conventional methods of controlling the diseases including the chemical methods proved not enough for total control of the disease with creating situation even worse for our surroundings, the application of PGPR and PGPF can play significant role to control the damage caused by *Fusarium*.

Keywords: mycotoxin, PGPR, PGPF, *Fusarium verticillioides*, *F. graminearum*, pathogens

1. Introduction

Maize is one of the most important cereal crop cultivated worldwide. It is popularly known as queen of cereals because it has highest population yield among the cereals [1]. Maize is the crop of diverse environmental conditions and now considered as one of the fastest growing cash crops in the world [2] and may play significant role to satisfy the ever increasing demand of world population. It is a multi-utility crop with major source of food, feed, fodder and industrial raw material which also provides huge opportunity to various stakeholders for crop diversification, value addition and employment generation [3, 4]. Maize plant is often induced by various types of naturally occurring pathogens like bacteria, virus, fungi and nematodes etc. and are detrimental to the yield and quality of grains and thereby subsequently affect the economy and threaten the food security around the globe [5]. Diseases are one of major obstacle in understanding the yield potential of maize. Among the disease causing pathogen in maize, fungal diseases caused by *Fusarium* spp. are most threatening not only to the plant but both animal and human's life are also equally intimidating. The contamination of human food and animal feed with mycotoxins produced by the *Fusarium* spp. incur various health issues in human and various livestock, such as equine leukoencephalomalacia, human esophageal and liver cancer etc. [6]. Despite of all these issues, the maize productivity over the years has been increased due to

green revolutionized availability of resistant variety and various synthetic fertilizers [7]. However, extensive and indiscriminate use of synthetic chemicals such as fertilizers and fungicides in order to improve the crop productivity has been very damaging not only to plant, man and animal but resulted adversely to many beneficial organism which includes rhizospheric microbial communities, pollinators, and competitors etc. [7, 8]. Therefore, application of microbes both PGPF and PGPR as biofertilizers is the only way out solve the problems which can play significant role in sustainable agriculture of maize plant without disturbing the surrounding environment. Along with application of biocontrol agent, diagnosis of the disease for proper identification is also very important part of eradication of pathogen. The proper identification of pathogen ensures the proper application of biocontrol agents. These microbes introduced to the soil in various form and by different techniques, interact with host plant and initiate cascade of reactions promoting the plant growth and impart defense responses [9]. Recently both PGPF and PGPR have attracted worldwide attention because of its eco-friendly nature, low cost and easy application. It is reported that many species of *Trichoderma*, *Penicillium*, *Phoma*, *Burkholderi*a, *Bacillus*, *Pseudomonas cepacia* are effective against *Fusarium* spp. through the production of various defense enzyme such as chitinase, peroxidase, phenylalanine ammonia lyase and β-1,3-glucanase etc. [9–12]. In addition, the plant growth promoting fungi and plant promoting bacteria are well known for plant growth promoting activity through the production of plant growth hormone like IAA, cytokinins, gibberelins; siderophore secretion, hydrocyanic acid (HCN) production, phosphate solubilization and nutrient uptake which indirectly boost the host plant for the defense against pathogen [13].

2. Fusarium disease of maize

Fusarium is considered as a most devastating agent among prevalent fungus on maize, particularly in USA, Europe, Africa, Asia and Australia [14]. It damages the host plant severely causing decrease in quality and productivity. *Fusarium* spp. are ubiquitous mostly soil borne pathogen which affect the plant development throughout the cultivation period. Infection of maize plant with *Fusarium* spp. such as *F. monilifrme* J. Sheld. (=*Fusarium verticillioides* (Sacc.) Nirenberg), and *F. roseum* f. sp. *Cerealis* "Graminearum" (Syn. *F. graminearum* Schwabe, group II), are believed to be responsible for the diseases like stalk rot, ear rot, seed rot, root rot and seedling blight of maize [15–17]. The different strain of *Fusarium* such as *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. graminearum*, *F. oxysporum* and *F. temperatum* are important pathogens involved in seedling diseases which interfere the seed germination and emergence and hence affect the seedling development [18, 19]. Brown discolouration of the seedlings, light yellowish discolored and stunted seedlings are major symptoms of seedlings [20]. Seed rot is another important disease occurs in weak and damaged seed which become easily susceptible to the attack of soil and seed borne *Fusarium* spp. Stalk rot is one of the most common and dominated diseases in maize characterized by tan to pink or salmon discoloration and disintegration of the pith caused by many species of *Fusarium* such as *F. graminearum*, *F. culmorum*, *F. verticillioides*, *F. proliferatum*, *F. equiseti*, *F. avenaceum*, *F. cerealis*, *F. poae*, *F. subglutinans* and *F. temperatum* [18, 21]. *The Fusarium spp.* belonging to the *Gibberella fujikuroi* species complex (GFSC) and *F. graminearum* (*Gibberella zeae*) like *F. moniliforme*, *F. temperatum* and *F. subglutinans* are very much associated with stalk rot disease in maize [22]. The common symptoms of stalk rot disease of maize include reduced growth, rotted leaf sheaths and internal stalk tissue and brown streaks in the lower internodes whereas at its maturity, it develops pink to salmon discoloration of the internal stalk pith tissues [23]. The *Fusarium* stalk rot disease may cause premature death of host plant hampering the nutrients and

water translocation to leaves and ears. The infected maize plant often wilt and appear from a light to a dull color and lower stalk dries (**Figure 1A**) with pith tissues disintegrating to a shredded appearance [24]. Tan to dark brown discolouration of the lower internodes and pink to reddish discolouration of the pith tissue are the distinct symptoms of stalk rot caused by *F. graminearum* whereas for *F. verticillioides*, brown streaks appear on the lower internodes and the rotted pith tissue may be whitish-pink to salmon in color [24]. The systematic and successful infection of seed and root gradually extends towards the internodes, stalk and ear contributing more and more diseases in plant [15]. Among *Fusarium* maize diseases, ear rots significantly contribute in loss of both quality and quality of the yield. Ear rot disease is basically concerned with corn ears and *F. moniliforme* which predominantly now known as *F. verticillioides*, has been reported as causal organism of the disease [25]. *Fusarium* ear rot has been reported as most common disease of maize in United States [25]. *F. temperatum*, a closely related species to *F. subglutinans* and reported form maize in different countries, was recently identified and described as a new pathogen causing ear rot in European maize [26, 27]. *F. graminearum* causing ear rot disease is characterized by a pinkish colored mold [24]. Typical symptoms of *Fusarium* ear rot caused by *F. verticillioides* include [28, 29]: (1) tan to brown discolouration or white or light pink mold on random kernels; (2) limited ear areas or groups of kernels scattered over the ear (**Figure 1B**). Symptomless kernel infection was also observed through systemic growth of *Fusarium verticillioides* from infected seed, roots or stalks through ear with peduncles maize [20]. Kernel and stalk are not only the susceptible parts of the maize plant to the *Fusarium* spp. but infections may also occur on foliar parts like leaf sheaths and around the blade-sheath boundary and husks, whereas the leaf blades appear visually unaffected throughout the vegetation period [20]. Comparative analysis of leaf sheath and leaf blade contamination with DON (mycotoxin) showed that higher concentrations of DON was in leaf sheath indicating that leaf infection by *Fusarium* spp. may move from primarily infected leaf sheath into the leaf blades [30]. The airborne spores of the fungus during its reproductive stage contributes its richness to maize field [31]. Further, *Fusarium* spp. also infect the husks, leaf sheaths and around the blade-sheath boundary with appearance of whitish mycelium and/or pink spore layers, reddish discoloration zones and necrotic lesions on husk, Leaf sheath and blade/sheath boundary [20].

Figure 1.
Major diseases of maize caused by Fusarium *spp.; (A) stalk rot disease and (B) ear rot disease.*

3. Source of inoculum and infection pathway

Many *Fusarium* spp. have been reported to cause the various diseases in maize but the most devastating fungal agent for ear rot and stalk rot diseases is *F. verticillioides* [32, 33]. Ear rot and stalk rot diseases are two most important *Fusarium* disease of maize occurs worldwide. The significance of these diseases has been witnessed by the world for many decades. Maize plant residues present in nearby fields is the primary and important source of inoculums for infections of maize plant [34]. Many *Fusarium* spp. successfully survive on maize crop residue or in the soil as mycelium or other structures like *F. graminearum* produces chlamydospores and *F. verticillioides* (reported as *F. moniliforme*) can produce thickened hyphae capable of colonizing senescent tissues of the host plant [24, 35]. *F. verticillioides*, *F. subglutinans* and *F. proliferatum* produce large numbers of microconidia and macroconidia on crop residues, and may act as the most important inoculum for *Fusarium* ear rot and symptomless kernel infection [25]. Though *Fusarium* spp. are commonly seed borne but the role of seed as an inoculum source for further infection has always been a matter of controversy [36]. According to Cotten and Munkvold, the surface residues may be the potential reservoir of recolonization and spore production for airborne inoculums contributing significantly in spreading of the disease into the next vegetation period [16]. *Fusarium* spp. can enter the host plant through different pathways and starts primarily from root infection, through stalk nodes or through injuries in the stalk made by various biotic agents, silk infection and systematic spread after root penetration [24, 37, 38]. In stalk rot disease, the *Fusarium* spp. enter the stalk systematically after the successful colonization of root through various ways such as seed transmission, young leaf sheath and via wounds created by hail or insects [33, 39]. At maturity, both root and lower stalk lose their metabolic activities weakening the plant defense system against infection [40]. The other factors such as drought, high plant density, leaf diseases, and corn borer attacks decreases photosynthesis rate in the host plant and may contribute in stalk rot disease development [40]. The major infection pathways taken up by most *Fusarium* spp. for maize ears infection is via silk which occurs severely at early stage of silk development [41]. There are three major entry points for ear infection: (1) landing and germination of fungal spores on the silk and moving down the silk to infect the kernels and rachis; (2) through injuries made by biotic agents and hail; (3) through systematic infection of *F. verticillioides* [24]. The important factors which appears to be affecting the range of different species of both ear and stalk rot infection are temperature and moisture [42]. However, the timing and significance of infection pathways may vary from one geographical region to another depending upon the weather conditions and occurrence of biotic agents. *F. graminearum* and *F. culmorum* are reported to cause the ear rot infection at low temperatures and high precipitation whereas *F. verticillioides*, *F. subglutinans* and *F. proliferatum* are responsible at high temperatures and dry conditions [25, 43].

4. Fusarium associated mycotoxin and its toxicity

Mycotoxins are low-molecular-weight secondary metabolites produced by various fungal group specially *Fusarium* spp. and are not only toxic to plant causing serious diseases in them but also significantly harmful to human and animals [44]. *Fusarium* is one of the most important plant-pathogenic fungi producing most important mycotoxin. Some of the emerging mycotoxin produced by the *Fusarium* includes: trichothecenes, zearalenones, fumonisins, and moniliformin. These mycotoxins are naturally found to occur in plants and its products all over the world. The

mycotoxins namely: fumonisins, beauvericin, fusaproliferin, and moniliformin produced by the strains of *F. verticillioides*, *F. proliferatum*, and *F. subglutinans* are commonly associated with maize ear rot disease [45].

Fumonisins (FUMs), especially FUM B1 (FB1) produced by *F. fujikuroi* species complex in warm climate are extremely toxic and carcinogenic to human causing liver cancers and human esophageal [46–48]. FB1 is also reported to have toxic effects in animals and several organs like kidney, liver, lungs, and nervous and cardiovascular systems [44, 49]. FUMs also exert its toxicity by causing wilting, chlorosis, and necrosis in maize and also interfere with shoot and root growth of the plant [44, 50]. It is evident that FUM phytotoxicity induced some symptomatic diseases in maize seedlings when inoculated with *Fusarium verticillioides* [51]. Trichothecenes (TRIs) are sesquiterpenoids with a tetracyclic ring system, categorized into type A to type D inhibits protein synthesis in eukaryotes and suppress or stimulate immune system [49]. They also produce some common effects on livestock like changes in neuroendocrine, hepatological and gastrointestinal systems; gaining of weight and feeding reduction [52]. Most TRIs have also been reported to produce phytotoxic effects. Reduced seed germination; stunting of coleoptiles, roots, and shoots; chlorosis; wilting; and necrosis are the most common toxic effect of TRIs [52]. Zearalenone (ZEA) produced by the *F. graminearum* and the *F. incarnatum-equiseti* in maize is effectly contaminated to its product and produce severity in their various consumers [44, 53]. Various species of *Fusarium* infecting maize have been reported with some of the emerging mycotoxin (**Figure 2** and **Table 1**).

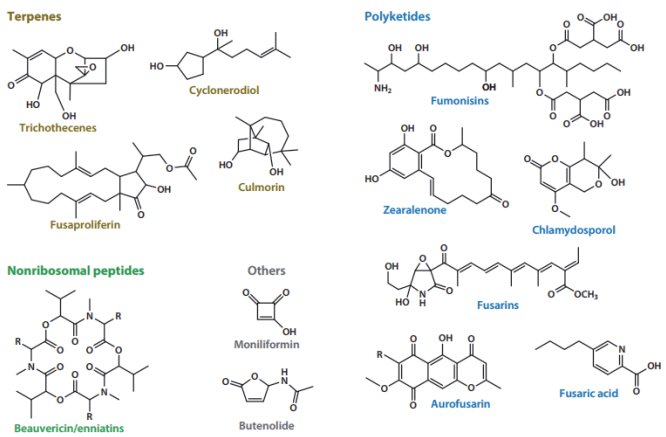

Figure 2.
Chemical structure of some important mycotoxin produced by Fusarium *spp. [44].*

Mycotoxin	Pathogen	Host	Reference
Beauvericin	*F. moniliforme*, *F. subglutinan* and *F. proliferatum*	Maize	[54, 55]
Moniliformin	*F. subglutinans*, *F. proliferatum*, *F. avenaceum* and *F. verticillioides*	Maize	[17]
Nivalenol	*F. graminearum*, *F. culmorum* and *F. crookwellense*	Maize	[56]
Fusaproliferin	*F. subglutinans* and *F. proliferatum*	Maize	[17]
Fusarin and fusaric acid	*F. graminearum* and *F. verticillioides*	Maize	[57]

Table 1.
Some of the emerging mycotoxin produced by Fusarium *spp. in maize.*

5. Diagnosis of pathogen

Earlier detection of plant pathogens is very important for plant health certification and to conduct the disease management appropriately [58]. The detection and enumeration of disease causing pathogen have always been challenging issues over the years. The environment form which they are originated, pose difficulties in identification, isolation and quantification of pathogen. Developing the accurate and effective detection methods and assay is very challenging for the pathogen like *Fusarium* spp. as they exists as multiple species complex, different pathogenic profiles and virulence levels to the host [59]. Over the years, several techniques and methods have been developed in order to detect and identify disease causing pathogen in crop plants. Some of the techniques widely used in identification of pathogen are morphological based identification; Immunological assay and PCR base methods. The morphology based identification of any plant pathogen is the first and one of most difficult steps in the direction of proper identification of disease causing pathogen in plants. The identification of fungal pathogen isolates based on morphological characteristics of culture is still in use for many laboratories. Since many *Fusarium* spp. offer species complex instead of single species and look similar in many aspects, it is always challenging for proper identification of the pathogen and hence mere morphology based identification may not work good for complete identification and may lead to wrong identification for sure. It is very difficult, time consuming method and requires experts [60]. Although morphological identification is not sufficient for proper identification of the pathogen, the observable morphological characters may give us great amount of information from the culture they grow [61]. Immunoassays technique for early detection and precise identification of pathogen has been significantly increased following the development of enzyme-linked immunosorbent assay (ELISA) and monoclonal antibodies with greater sensitivity and specificity compared to morphological based methods [62]. Serology-based technique can be used to detect fungi present even in low quantities on plant tissues at an earlier stage of disease development. The earliest serological techniques used, were polyclonal antisera from immunized animals by centrifugation of clotted blood [63]. This method is further refined to a serum fraction in classical enzyme-linked immunosorbent assay (ELISA) with IgG as dominant which is obtained by ammonium sulphate precipitation, then passage over an ion exchange cellulose column is followed [64]. Now monoclonal antibodies in plant pathology are more routinely used but polyclonal antisera are also in use in this kind of techniques. Detection or quantification of binding of the diagnostic antibody with the target antigen is the principal aim of all the serological techniques used in plant pathology and double immunodiffusion techniques, indirect immunofluorescence assays and ELISA are most common used techniques. Techniques using antibodies are now suitable for both laboratory and field conditions and can identify pathogenic strains within species in very short span of time. In recent years, the PCR based identification techniques is most preferred and widely used technique among others due to its greater speed, specificity, sensitivity and reliable reproducibility. Polymerase chain reaction (PCR) is one of the greatest achievements of molecular biology. PCR synthesizes DNA and nucleic acid fragments can be specifically replicated in a semiconservative way. Currently, with the help of PCR-based technique, the taxonomic status of any fungal isolates can easily be determined. This technique has the ability to differentiate fungi species which are very closely related and morphologically similar in nature e.g. *F. proliferatum*, *F. temperatum*, and *F. verticillioides* belonged to *Fusarium fujikuroi* species complex (FFSC) [61]. PCR-based molecular methods along with sequencing of ribosomal DNA is now being successfully used to detect and identify the richness of the species from

different environments [65]. The ribosomal internal transcribed spacer (ITS) region of nuclear rDNA from gDNA of the *Fusarium* isolates is amplified through PCR by using ITS1 and ITS4 primers followed by analysis of ITS sequences based on a ClustalW Multiple alignment using suitable BioEdit software [66, 67]. The search for homologous sequences is then conducted using Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) for exact identification of fungal pathogen [68].

6. Disease control through sustainable approach

The significant problems caused by *Fusarium* spp. in maize crop production worldwide include reduce in crop quality, decrease in yield, and higher production costs. Once soilborne *Fusarium* spread in the field, it is very difficult to control. The one of the major reason for not being able to control the *Fusarium* diseases of maize including ear and stalk rot diseases is, its nature of being survived in crop residue for longer period of time and being endophytic, many *Fusarium* spp. remain away from the contact of chemical control [16]. Currently, in order to control the soilborne pathogens the conventional methods of controlling like soil disinfection using fumigants, hot water, or solarization, or using resistant cultivars are very popular and even the chemical fertilizers are in great use [69]. However, the efficacy obtained with these treatments is not up to the mark and found less effective than expected. Many of these methods have been found to be very harmful not only to the plants only but man; animal and associated beneficial microbial communities are severely affected. Therefore, there is an urgent need of finding such an alternative option which could be eco-friendly and sustainable. There are many microorganisms dwelling in the rhizospheric zone of the host plant which significant not only in controlling the soilborne diseases causing pathogen but their role in plant growth promotion is quite commendable. These biocontrol agents have been proved to be eco-friendly, less expensive and more sustainable tools for disease management. Biocontrol agent use different mode of action in order to control the disease which include nutrient competition, antagonism, and production of toxic metabolites and induced systemic resistance (ISR) through the production of defense enzymes. Many microorganism like *Trichoderma* spp., *Penicillium*, *Bacillus* spp., *Rhizobium* and *Pseudomonas* spp. have been reported to function as plant growth promoting fungi (PGPF) and plant growth promoting rhizobacteria (PGPR) in addition to their potentiality to boost defense mechanism [70, 71]. Among the fungi, *Trichoderma* is often considered as universal biocontrol agent due to it extra ordinary function such as mycoparsitism, antibiosis, production of extracellular enzyme, competition for space and nutrients etc. [72].

6.1 Antibiotic production

Antibiosis, a kind of interaction takes place between two organisms when one produces antimicrobial metabolites called antibiotics that directly check the growth and metabolism of the other organism. Antibiotics are low molecular weight toxic organic compound produced by many organisms in order control the growth of pathogen. It is assumed to be one of most effective measures having antagonistic activity against wide range of phytopathogen. Bacteria can either produce single antibiotic and toxin or can produce them in multiple numbers. The antibiotic and toxin produce by bacteria include pioluteorin, pyrrolnitrin, hydrogen cyanide (HCN), oomycins, polymyxin, circulin, colistin and tensin etc. [73]. Bacteria and fungi of various genera, such as *Bacillus* spp. *Microsphaeropsis* sp. *Trichoderma*

harzianum and nonpathogenic *Fusarium* spp. have been identified as microbial antagonists of *Fusarium* spp. through the mechanism of antibiosis elicited by a wide range of antifungal metabolites, including antibiotics [74]. The ability to produce multiple classes of antibiotics by various group of microorganism enhances the biocontrol activities against the phytopathogen. Microbiotacontaining fungi belonging to genus *Trichoderma* are found eliminate plant pathogens by producing specific and nonspecific antibiotics such as trichodemin, trichodermol, harzianun and harzianolide etc. [75].

6.2 Production of extracellular enzyme

Cell wall-degrading enzymes produced by biocontrol strains of bacteria and fungi have a definite role in restricting the growth of various pathogenic fungi including *Fusarium*. The exracellular enzyme such as chitinase and β-1,4-glucanase etc. interferes with fungal growth by lysing and degrading the cell and cell wall of the pathogenic fungi. *Trichoderma* spp. are very effective biocontrol agents because of their powerful extracellular lytic enzymes activity against fungi through lysis of cell walls [10]. Chitinases from bacteria and fungi are reported with fungicidal and insecticidal activities against *Fusarium* spp. showing extraordinary role in bio-control mechanism include *Streptomyces*, *Pseudomonas*, *Bacillus*, *Trichoderma* spp. and *Penicillium* spp. [71]. Various strains of *Bacillus* has been found to produce chitinase and glucanase for biocontrol mechanism against *Fusarium verticilloides* [76]. There is a report that *Trichoderma asperellum* along with other extracellular enzymes like chitinase and protease, produced β-glucanases against *F. graminearum* causing stalk rot of maize [77].

6.3 Competition for root niche and nutrient

Competition between pathogens and non-pathogens for nutrient resources is important for limiting disease incidence and severity. Rhizosphere is hotspot zone of microorganism and nutrient rich environment which provide a suitable platform for the interaction. Competition for these nutrients and niches is a fundamental mechanism by which beneficial microorganism both bacteria and fungi protect plants from phytopathogens. The interaction between them brings the beneficial microbes to control the disease causing pathogen. Soilborne pathogens, such as species of *Fusarium*, that infect through mycelial contact are more susceptible to competition from other soil and plant-associated microbes [78]. The nonpathogenic microorganisms extremely dependent on exogenous nutrient make them highly competitive with pathogenic microorganism. Competition for nutrient like carbohydrates in the nutrient rich environment in combination with competition for the limited amounts of nitrogen sources such as amino acids play the key roles in the antagonistic interaction [79]. Mycorrhizal fungi are also potential candidates for biocontrol through competition for space and nutrients by virtue of their ecologically obligate association with roots [80].

6.4 Siderophore production

Iron is one of the most common trace elements in nature required by almost all the living organism for their growth and metabolism. Siderophores are low molecular weight extracellular chelating compounds and have a great affinity for ferric iron that are produced by many microorganism like *Trichoderma* spp., *Penicillium* spp., *Bacillus* and *Pseudomonas* etc. in response to iron deficiency. They are secreted into the surrounding environment to dissolve iron minerals and hold it in a soluble form so that they can acquire them by diffusion and consequently enhance plant

development by increased uptake of iron [81, 82]. Solubilization and the competitive acquisition of iron under limiting conditions restrict the availability of iron to soilborne pathogen, subsequently limiting their growth [83]. Many *Fusarium* spp. are found to be inhibited by both PGPF i.e. *Trichoderma* spp. and PGPR i.e. *Pseudomonas* through siderophore mediating competition. PGPR such as *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* have been found to protect maize against *Fusarium verticillioides* [84]. Siderophores produced by *Pseudomonas* species (pyoverdine, pyochelin) has shown siderophore-mediated competition for iron and in the control of *Fusarium* [85].

6.5 Induced resistance

Rhizospheric microbes protect the plant not only through their antagonistic properties but also help the plant to defend itself from the pathogenic attack. The term induced resistance is meant for the induced state of resistance in plants triggered by various biological inducers and subsequent protection of non-exposed plant parts against future attack by pathogenic microbes of any kind. Induction of resistance can be local and/or systemic in nature depending on various factor such as types, source, and stimuli. There are two types of induced resistance namely SAR and ISR which provide long-lasting resistance against plant pathogens. Systemic acquired resistance (SAR) is mediated by salicylic acid (SA) and produced following pathogen infection and leads to the expression of pathogenesis-related (PR) proteins. PR proteins include enzymes which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death [78]. Induced systemic resistance is the process of active resistance against pathogen and is induced upon by colonization of beneficial microbes like PGPF and PGPR or infection by some specific pathogen. It does not rely on SA but depends on the pathways regulated by jasmonate and ethylene [86]. Pathogenic microorganisms trigger a wide range of defense mechanisms in plants through ISR. The major changes occurs in root of the host pant through ISR are: (1) Strengthening of epidermal and cortical cell wall; (2) increase in levels of defense enzyme such as chitinase, polyphenol oxidase, peroxidase, phenylalanine; (3) increase in phytoalexin production and (4) expression of stress related genes [80]. ISR extending up to the shoots from roots protects the unexposed parts of plants against pathogenic attacks by microorganisms in future [87]. The induced resistance is elicited by various beneficial and non-beneficial organisms and regulated by signal pathways, where plant hormones for example play a vital role in inducing the resistance which is regulated by networks of interconnected signaling pathways [88]. Several *Pseudomonas* spp. and *Bacillus* spp. participate in induced systemic resistance (ISR) in a wide range of plants against different pathogens [89]. The PGPR like *Pseudomonas aurantiaca* has been reported to induce the immunity in maize apart from growth promotional activities [90]. Many fungal biocontrol agents such as *Trichoderma* spp., *Penicilliumn simlicissmum* and *Phoma* spp. have also been found to elicit the induced systemic resistance [91].

7. Bioformulation

Pseudomonas spp. and *Trichoderma* spp. have many success stories as a part of bioformulation inducing disease resistance and plant growth promotional activities. Different organic and inorganic carrier materials may function for effective delivery of biocontrol agents. However, the concentrated formulation with potential biocontrol agents provided an extra advantage of smaller packaging with respect to storage and transportation, and low product cost as compared to other carrier

materials such as charcoal, vermiculite, sawdust and cow dung [92]. A talc-based formulation with *T. harzianum* was developed aiming at supply of concentrated conidial biomass of the biocontrol agent with high colony forming units (CFU) and long shelf life which significant increased the plant growth promotion [93]. The application of seed based bioformulation using *Pseudomonas fluorescens* not only reduced the disease incidence of *F. verticillioides* causing disease of maize but helped the plant to achieve the plant's growth [93].

8. Conclusions and future prospects

Maize is one of the main contributors to the economy and food security of the world. A Suffering of maize plant by the *Fusarium* is immense only to the plant but entire biotic community. World has witnessed the effect of *Fusarium* on maize plant and subsequent role in causing the diseases in man and animal. Complete control over the *Fusarium* causing disease in maize has not been possible till date and some of control mechanism like chemical control has made the situation even more critical by creating unhealthy environment. Therefore world must look and emphasize on biocontrol mechanism to ensure the security and healthy environment for the next generation. There is a huge existence of unknown microbes in the soil and therefore we must not rely on limited and known microbes but investigate sustainable potentiality of others which could play significant role in this regard. It is also very important to commercialize their production for mass application through sustainable way.

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Chapter 6

Importance of the Natural Incidence of the *Fusarium* Genus in Food Crops Established in Northern México

Julio César Ríos Saucedo, María Gabriela Ramírez-Valadez, Saúl Santana Espinoza, Maihualy Martínez-Fernández and Rigoberto Rosales-Serna

Abstract

The incidence of the *Fusarium* genus causing root rot is reviewed in crops showing high importance for food supply and to obtain regular income by farmers in the highlands of Northern México. Pathogen incidence was evaluated under field conditions in multiple sampling locations for common beans (*Phaseolus vulgaris* L.) and several chili peppers (*Capsicum annuum*) local cultivars (landraces and bred cultivars). Five commercial plots for registered and certified seed were also evaluated in common beans to be used in the 'seed refreshing program' implemented for the cultivar Pinto Saltillo, considered as the main variety sown in the highlands of México. High *Fusarium* genus incidence and its interactions with other fungi species, such as *Rhizoctonia solani* and *Pythium* spp., cause high losses in plant population, commercial yield and seed quality in food crops grown in Northern México. The natural incidence of plant disease caused by the *Fusarium* genus and its negative effect on crop survival and the reduction of commercial yield and seed quality is fully reviewed. Plant disease resistance, crop breeding and the influence of the environmental conditions were also considered.

Keywords: *Phaseolus vulgaris*, *Capsicum annuum*, root rot, commercial yield, seedborne pathogens

1. Introduction

The *Fusarium* genus includes several fungi species considered as the most important soil and seedborne pathogens causing plant diseases with high yield, quality and economic impact in several food and cash crops sown in the highlands of México [1]. The main effect of the *Fusarium* genus is the root and crown rot observed in vascular bundles of common bean (*Phaseolus vulgaris* L.), chili pepper (*Capsicum annuum* L.), maize (*Zea mays* L.) and other crops used as food, fodder and to obtain cash for urgent expenditures by the poor farmers in Northern México [2]. The main *Fusarium* symptoms in the plants are observed at the soil surface or

below the ground including damage in root crown and embryonic root, losses of root water-absorbing surface, leaf wilting and plant death [3, 4]. As a result, low plant densities are commonly observed in common bean and chili commercial plantings in most of the producing areas of Northern México [5, 6].

Under rainfed conditions losses in plant population are aggravated in years registering drought stress [7] after the common bean seedlings emergence. Plant losses are also observed under irrigation [8] when high temperatures and fast drying of the soil surface are registered, mainly after plant emergence (V_1) and the primary leaves unfolded (V_2) stages in common beans [9] and the second week after chili seedlings transplant in cultivated soil. Under those conditions, no post-embryonic adventitious root growth and plant death are observed due to severe damage in the embryonic root caused by the fungi complex (*Fusarium* spp., *Rhizoctonia* spp., and *Pythium* spp.) [10].

Plant losses in the early stages of growth in common bean and chili are considered one of the main causes of reducing yield and the quality of the commercial product (seed and chili pepper: fresh, dried, or processed fruits) [5, 11–13]. Decrement of the income is also observed by farmers, considering that low-input agriculture is the best option for reducing risks in crop production thus obtaining low yields. The actual measurement of the incidence and severity levels of plant pathogens causing root and crown rot, as well as diseases in the aerial plant organs is necessary to establish control strategies according to agroecological and sustainable agriculture. Identification of plant disease agents is the key to the development of effective control and management strategies [14]. Actual evaluation of disease problems related to the *Fusarium* genus in the state of Durango was included in this study regarding the importance of common bean and chili peppers as important food-producing crops.

2. Common bean

Common bean is an important cash crop used as a food, nitrogen-fixing plant and organic matter source [15], thus helping to reach sustainability in the agriculture performed under drought-prone and irrigated areas in the Mexican highlands of Northern México, including the states of Zacatecas, Durango and Chihuahua. In this area near 1.6 million of hectares are annually sown and more than 1.0 million of tons of grain are produced, at a rate of 0.69 t ha^{-1} [16]. Common bean cultivars Pinto Saltillo (improved) and Negro San Luis (landrace) are the most important varieties, according to the planted area and grain volume produced. Sowing recommendations include 35 to 50 kg ha^{-1} of seed [17] to obtain plant densities ranging from 100,000 to 120,000 plants ha^{-1}. In despite of recommendations low plant densities (<80,000 plants ha^{-1}) are very common in commercial plantings through the common bean-producing areas of Northern México, thus lower seed yield is also observed across locations and years [2].

Reduction in plant densities reached values lower than 20% of the recommended levels, and the lowest levels (12,500 plants ha^{-1}) were related to the lowest seed yield [5]. Plant densities reduction in early stages of crop growth were related to the low seeding rates, mechanical soil impedance and seedlings death caused by the fungi complex including *Fusarium solani* f. sp. *phaseoli*. Identification and predominance studies of pathogenic fungi are important to establish control strategies to reduce disease problems. Studies on fungi genetic and pathogenic diversity are also important for efficient disease control in several crop-producing areas of Northern México. Several strategies were used for fungal disease control including

crop genetic breeding, crop rotation and other modern agroecological and chemical management technologies.

2.1 *Fusarium* natural incidence

Twenty root samples were taken during the 2007 growth cycle in 15 common bean commercial plots distributed across "Los Llanos" de Durango and the Valleys of Poanas, Guadiana and Canatlán [10]. Fungi genus predominance was recorded using presence or absence in each root sample. Seventy-five fungi strains were isolated mainly belonging to three genera as follows: 54% for *Fusarium* sp., 10% *Macrophomina* and 2% *Pythium*. *Fusarium* resulted in a genus widely distributed, present in all the sampled locations and within genus three species were identified as *Fusarium oxysporum*, *F. solani* and *Fusarium graminearum* [10].

The predominance of *Fusarium* was observed in 93% of sampled locations and in 75% of the samples was the only genus of fungus found, affecting all the seed commercial classes (pinto, shiny black and flor de junio), while *Macrophomina* was associated mainly with pinto cultivars [10]. Results corroborated previous findings which demonstrated a close relationship between the *Fusarium* genus and common bean root rot in the state of Durango [1]. Systematic studies for fungi phenotypic, pathogenic, and genetic variation need to be implemented to select efficient, sustainable and agroecological disease control methods.

Another study was performed in Durango during 2020, including seven commercial plots at two important common bean producing areas (**Table 1**). The importance of *Fusarium* fungus was corroborated and variation in presence values from 50 to 100% were obtained. Other fungi genera were found at different levels of presence including *Rhizopus* (0–40%), *Pythium* (10–30%), *Erysiphe* (10–20%) and *Cercospora* (10–20%). The *Fusarium* fungus remains as the major plant disease problem in Durango, where integral control programs are necessary including crop genetic improvement, crop rotation, and other agroecological control methods.

Preventive chemical control and qualified (registered and certified) seed use are also considered. *Fusarium* control is an important issue due to some fungal species on stored common bean can also release mycotoxins (fumonisin) which causes human mycotoxicoses upon consumption, esophageal cancer and interferes with sphingolipid metabolism [18].

		Fungi genera				
Municipality	**Plot**	***Fusarium***	***Rhizopus***	***Pythium***	***Erysiphe***	***Cercospora***
Durango	1	50	40	10	20	20
	2	100	40	30	10	20
	3	80	40	20	10	20
[1]G. Victoria	[2]J. G. R.	60	30	20	10	20
	A. A.	70	30	20	20	10
	I. A.	60	0	30	10	20
	C. C.	70	0	10	10	10

[1]G. Victoria = Guadalupe Victoria.
[2]J. G. R. = José Guadalupe Rodríguez, A. A. = Antonio Amaro, I. A. = Ignacio Allende and C. C. = Calixto Contreras.

Table 1.
Isolation frequency of pathogen fungi related to common bean root rot at different municipalities of Durango, México. 2020.

2.2 Genetic improvement

Few systematic studies have been implemented in Durango for root diseases, mainly due to difficulties observed for plant root extraction, destructive sampling methods, and laboratory requirements for pathogen isolation and conservation, as well as for selecting crop-resistant germplasm. Studies concluded that low genetic diversity was observed for resistance in the plant pathogen-host represented by multiple common bean cultivars belonging to different genetic races and variable seed sizes and color. Sources of resistance against *Fusarium* wilt were identified in flor de mayo (pink) germplasm [19], mainly related to the Jalisco Race [20].

Low genetic resistance to *Fusarium* spp. has been identified in cultivated common bean populations, and the genetic resistance is quantitative [21] and hence, strongly influenced by the environment [22]. Other studies reported dominance in the control of the character with additive effects in common bean indicating that selection should be easy using efficient inoculation and selection methods [23]. In Durango, no direct selection was performed for *Fusarium* root rot in common bean, however, results indicated that resistance to FSP was more frequent in black beans [11]. In despite of the general observations, pinto seeded cultivars (Durango Race) showed capability for rapid adventitious root growth to maintain water absorption from the superficial soil layers and reduce losses in plant population.

2.3 Crop rotation

A common bean monocropping system is a common agricultural practice in most of the production areas in Northern México, thus aggravating problems and damage caused by *Fusarium* and other soilborne and seed transmitted pathogens. Crop rotations under rainfed conditions depend on the rain occurs during the May to August period. Early rains (May and early June) favor maize plantings while oats sowing is preferred when late raining periods (after middle August) are observed. Common bean is preferred to be sown when the rains are registered in late June to middle July. Under irrigation forage crops such as corn, sorghum, grasses, lucerne and oats are preferred due to pressure exerted by cattle farmers. Systematic crop rotations are required in Durango to reduce plant root and aerial pathogen problems in several crops and advances in sustainability could be also achieved by reducing water use by planting low water requirement crops.

2.4 Agroecological control methods

Trichoderma sp. showed high efficiency as a control agent for a wide range of aerial and soilborne plant pathogens and this trait makes it an excellent candidate for controlling saprophytic growth of *Fusarium* [24]. Some attempts were made to evaluate to control efficiency of this natural soil organism by reducing plant damages and yield losses caused by *Fusarium*. Results are considered ambiguous and *Trichoderma* use are not yet included in common bean crop management recommendations. Other options have been explored such as plant-based biopesticides [25] without actual use at the commercial level.

2.5 Chemical control

Disease chemical control starts with the seed treatment, but in most of the production areas in Durango the use of a fungicide is considered only in qualified seed production programs, which include several quality categories: basic, registered, certified and declared seeds [26, 27]. Most of the farmers consider chemical treatment as a

"fallacy" used only to justify the increment in seed prices without additional benefits for plant disease control under field conditions. Chemical products recommended in common bean seed treatment are: Metacaptan® (Captán + Metoxichloro), Thiram (Tebuconazole + Thiram), Terrazán [(Quintozeno-(pentacloronitrobenceno)], vitavax (Carboxín + Captán), Ridomil (Metalaxil-M) and Benlate (Benomyl).

The field visual evaluations showed some beneficial effects of the chemical seed treatment for increment the seedling emergence and plant survival at the early stages of the common bean development. Although systematic evaluation of subsequent fungicide effects on seedling and adult plant disease control and seed yield are necessary to reinforce the recommendations for its use. Several fungicides showing contact and systemic effects are recommended to reduce root rot incidence and severity, but results are not conclusive. Plant genetic resistance is preferred across the common bean production areas along México where several cultivars have been developed [28].

2.6 Qualified seed

Qualified seeds used in México for common bean commercial plantings are known as basic (foundation), registered (first generation certified), certified (second generation certified) and "enabled" seed. Standard seed treatment include fungicide (Metacaptan®, Thiram, Terrazán, Vitavax, Ridomil and Benlate), insecticide (Deltamethrin) and rhodamine as a colorant [26]. Effectivity on seed treatment needs to be evaluated due to the increase in the number of companies dedicated to seed production under several environmental conditions and variation in compliance with the regulations. In 2021, five seed lots from different sources were evaluated considering quality, which includes the genetic, physiological, physic, and sanitary traits (**Table 2**). The standard germination test is the most used probe to evaluate the physiological quality of a seed lot.

The pathogen attack on seeds is one of the factors that leads to the physiological quality loss, reducing the germination rate and the vigor of the seed lots, which end up precluding the final stand of the crop, resulting in productivity and economic losses to the farmer. Fungi are considered as the most important among pathogens due to the higher number of species and the damage caused both in yield and seed quality [29]. The mixture containing Carbendazim + Thiram in its composition are efficient in the control of pathogens regardless of the application time of products [29].

The sanitary quality of the seed used in México needs to be evaluated to reduce problems during the seed germination and seedling emergence periods, mainly under field conditions. Some chemical products were identified for their efficient control of different fungus although some phytotoxicity effects were also observed and delayed protection by fungicide controlling *Fusarium* on seeds [29]. In 2021, five

Seed Lot (origin)	Category	Year of production	Reception date	Amount (kg)
1. Chihuahua 1	Registered	*SS-2020	04/12/20	3000
2. Chihuahua 2	Registered	SS-2020	15/12/20	3000
3. Durango 1	Certified	SS-2016	15/02/2021	10,030
4. Sinaloa	Registered	AW-20	30/03/2021	3000
5. Durango 2	Registered	SS-2020	15/06/2021	9000

**SS = spring–summer growth cycle; AW = autumn-winter growth cycle.*

Table 2.
Reception data of five qualified seed lots to be used in reinforcing Pinto Saltillo common bean commercial plantings in Durango, México.

seed commercial lots (**Table 2**) were evaluated according to the physiological quality tests at the INIFAP's Valle del Guadiana Experiment Station, located in Durango, Méx. Standard germination test was performed, using a soil-based substrate, and considering the SNICS (Servicio Nacional de Inspección y Certificación de Semillas) recommendations for sample size (30 samples) in each seed lot. The seed lot number 4 from Sinaloa registered the highest emergence level (96%), reaching that value in the shortest period (8 days after planting; DAP); while, lot number 3 (from Durango) showed the lowest emergence value (65%), reached at 12 DAP (**Figure 1**).

Reductions in seed germination levels were related to the production environment and storage conditions and duration. Other negative factors were mechanical damages caused during the harvest-threshing and seed cleaning processes, as well as the seed fungi load and chemicals used for the seed treatment [29, 30]. Seedlings wilting was also observed during the germination test due to soil infestation and seed contamination by fungi. Therefore, seed studies for soil and seedborne pathogen load were performed under controlled laboratory conditions.

2.7 Seed health tests

Pinto Saltillo is an improved common bean cultivar showing high yield and disease susceptibility in aerial (Common Bacterial Blight) and root zone (*Rhizoctonia* spp., *Fusarium* spp., and *Pithyium* spp.). Most of these diseases are seedborne and the infestation/contamination of the seed may occur during harvest-threshing activities, processing and handling. The pathogen may, thus, be carried with the seeds in three ways: Admixture (pathogen are independent of seed but accompany them), External (pathogen present in seed surface as spores, oospores and chlamydospores) and internal (pathogens establish within the seed with the definite relationship with seed parts) [31]. The pathogen *Fusarium* is soil as well as seedborne in nature and the colonization percent of *F. solani* was highest as compared to other isolated fungi. Seed germination rate was also reduced (50%) in soil infested with *F. solani* where seedlings mortality reached 93.3% [32].

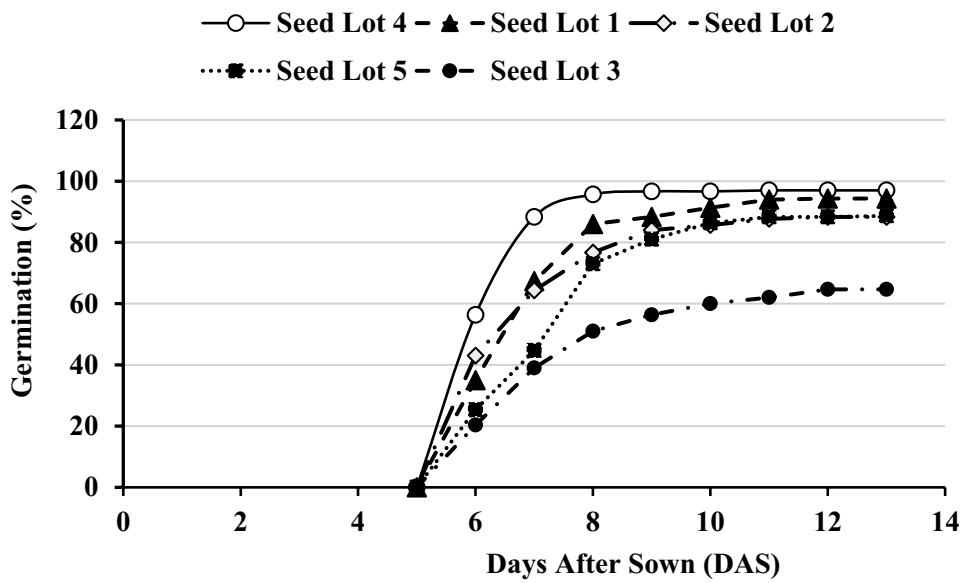

Figure 1.
Germination standard test performed in five qualified common bean seed lots obtained in México. 1) Chihuahua 1, 2) Chihuahua 2, 3) Durango 1, 4) Sinaloa y 5) Durango 2.

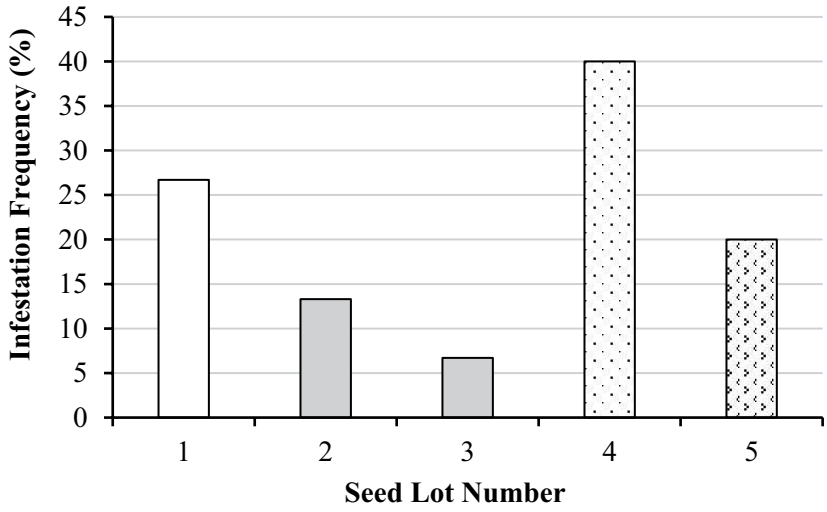

Figure 2.
Fusarium fungus infestation frequency observed in seed lots from different states to be used in Durango. 1) Chihuahua 1, 2) Chihuahua 2, 3) Durango 1, 4) Sinaloa y 5) Durango 2.

Agar plate is considered the most common method used for identification the of seedborne fungi. In 2020, *Fusarium* presence in Pinto Saltillo seed lots from different origins (**Table 1**) was determined by triplicate placing seeds onto sterile agar media (Potato Dextrose Agar: PDA) to encourage the growth of the fungus [33]. Variation of the infestation frequencies was registered among seed lots from different seed sources from México (**Figure 2**). High frequencies (40.0%) were detected in Pinto Saltillo seed lots produced in Sinaloa during the Autumn-Winter (2020–2021) growth cycle where several *Fusarium* hospedant were cultivated (chickpea, tomato, common beans and maize) [2].

Fusarium isolates from Sinaloa, Méx., showed differences in aggressiveness; and *F. falciforme* was the most aggressive compared to *F. oxysporum* [34], and isolates of both complexes triggered similar aerial symptoms of yellowing and darkening of the vascular tissues in tomato plants. But only *F. falciforme* isolate triggered necrosis in the plant crowns [34]. Seed lot 3 showed low frequencies for *Fusarium* fungus incidence (6.7%), mainly due to the longest storage period since was produced in the 2016 Spring–Summer growth period. Long storage period reduced pathogen fungus load but low seed germination and seedlings damage (injured leaves) were also observed.

3. Chili peppers

In México, generic name of chili pepper (chile; *C. annuum* L.) is used to denominate several plant cultivars mainly known with a local names such as: chile ancho, jalapeño (processed chipotle), serrano, mirasol (dried guajillo), and pimiento morrón reaching the 70 and 80% of the national production [35]. Chili pepper is one of the most important vegetable crops used in México as condiment and food flavor and its also considered as an important cash crop [36] grown in several production areas in Northern México, providing additional income to the farmers.

In the Northern highlands of México, the planted area for fresh fruit harvest of chili pepper in 2020 reached 37,440 ha in Zacatecas, 30,772 ha in Chihuahua and 4,136 ha in Durango [16]. In Durango, several chili cultivars and landraces are

planted [jalapeño, poblano-ancho, puya, mirasol-guajillo, árbol, cola de rata and tornachile (chile güerito)]; while in Chihuahua and Zacatecas, the jalapeño, serrano and habanero cultivars are preferred. The highest chili production is obtained in Chihuahua (722,937 t) where the yield rate is 24.0 t ha^{-1}. The state of Zacatecas produces 458,943 t and the yield average is 12.4 t ha^{-1}. In the state of Durango the chili production overpasses 48,035 t and the lowest yield at the North Central region of the Mexican highlands is obtained (11.6 t ha^{-1}) [16].

Chili crop management system includes seed obtention from dried fruits, sowing and nursery growth (almácigo), and transplant under field conditions, using rows 0.81 to 1.20 m apart [35]. Modern management techniques include the use of mulch and drip irrigation [37] to increment yield and water productivity. Several plant pathogens are observed in chili plantations established in Durango, causing phytosanitary problems, low yield and reduced fruit quality; as well as generalized plant or whole plot losses. Plant pathogen problems include viruses (Cucumber Mosaic: CMV, Potato Y: PYV, Alfalfa Mosaic: AMV, Tobacco Mosaic: TMV and TEV), fungi (*Phytophthora capsici*, *Rhizoctonia solani*, *Fusarium* spp., *Pythium* spp.) [12] and bacteria (*Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*) [38].

The pathogenic syndrome known as chili plant wilting 'Secadera' (CPW), is the most important disease in all the producing areas of México, causing total yield losses (100%) and the planting area was reduced by 60% in some states [39]. CPW is mainly caused by the obstruction of the vascular bundles provoked by the phytopathogen fungi infection (*Fusarium oxysporum, Rhizoctonia solani*, and *Phytophthora capsici*) in roots or root-crown [34]. The plant pathogen, mainly *Fusarium*, relative importance need to be evaluated due to its alimentary, health, economic and social implications.

3.1 *Fusarium* natural incidence

Root and crown samples were taken when 'Secadera' (Damping-off and CPW) symptoms (symptomatic plants) were observed at the main chili-producing areas in the state of Durango.

3.1.1 Study 1

Eleven municipalities were included (**Table 3**) and random sampling included 26 commercial plots established under irrigation in each of the chili pepper production areas across the state of Durango where temperate and warm climates are registered. Plots were georreferentiated for map construction (**Table 3**). Direct sampling was made in plots showing typical Damping-Off and CPW symptoms, such as: yellowing and wilting in upper leaves, wilting symptoms in all parts of the plant; leaves showing dark-green color remaining attached to the plant, root crown narrowing and plant death. Samples consisted of 10 plants in each plot, which were dissected and tissue samples were taken in the root crown and embryonic root. Samples were transported in paper bags with an identification label, including municipality, location, geographic coordinates and crop cultivar, then were sun-dried and stored at room temperature until processing.

3.1.2 Study 2

Seven chili pepper sampling sites were included at two municipalities in the state of Durango (**Table 4**), plots were mainly established under irrigation in the temperate climate regions. Plots were georreferentiated for map construction (**Table 4**). Direct sampling was made in plots showing typical Damping-Off and CPW symptoms. Samples consisted of 10 plants in each plot which were dissected,

Sample number	Municipality	Plot	Geographic localization	Cultivar local name
1	Simón Bolívar	Simón Bolívar	N 24° 45′ 42.0″; W 103° 10′ 51.5”	Ancho
2	Simón Bolívar	Flores Magón	N 24° 44′ 43″; W 103° 10′ 29.0”	Ancho
3	Lerdo	El Refugio	N 25° 25′ 59.0″; W 103° 44′ 57.3”	Guajillo
4	Lerdo	21 de marzo	N 25° 26′ 45.2″; W 103° 45′ 13.0”	Guajillo
5	Mapimí	Perimex	N 26° 32′ 36.6″; W 104° 08′ 15.8”	Jalapeño
6	Nazas	Francisco Sarabia	N 25° 18′ 12.6″; W 103° 58′ 20.5”	Guajillo
7	Nazas	Agustín Melgar	N 25° 16′ 25.3″; W 103° 58′ 20.5”	Guajillo
8	Nazas	Lázaro Cárdenas	N 25° 16′ 25.0″; W 104° 02′ 43.8”	Guajillo
9	Nombre de Dios	Francisco Munguía	N 23° 47′ 33.6″; W 104° 06′ 15.2”	Puya y Árbol
10	Peñón Blanco	J. Agustín Castro	N 24° 38′ 51.0″; W 103° 56′ 01.1”	Ancho
11	Poanas	Dago 1	N 23° 58′50.2″; W 104° 03′ 54.0”	Mirasol
12	Poanas	Pozo 12	N 23° 59′ 29.3″; W 104° 04′ 00.7”	Ancho y Puya
13	Poanas	UTP 1	N 23° 56′ 26.7″; W 104° 02′ 56.8”	Ancho y Puya
14	Poanas	UTP 2	N 23° 55′ 53.9″; W 104° 02′ 57.8”	Ancho y Árbol
15	Poanas	UTP 3	N 23° 56′ 26.7″; W 104° 02′ 56.8”	Ancho
16	Poanas	Pilares	N 23° 52′ 24.7″; W 104° 02′ 40.2”	Puya
17	Rodeo	El Parián	N 25° 09′ 45.2″; W 104° 32′ 40.2”	Ancho
18	Rodeo	Primo de Verdad	N 24° 54′ 52.7″; W 104° 28′ 5.5”	Ancho y Cola de Rata
19	Rodeo	La Cuesta	N 25° 01′ 47.7″; W 104° 28′ 40.9”	Ancho
20	S. Juan del Río	El Crucero 1	N 24° 46′ 58.4″; W 104° 30′ 56.9”	Ancho
21	S. Juan del Río	El Crucero 2	N 24° 46′ 51.3″; W 104° 31′ 14.8”	Ancho
22	S. Juan del Río	Francisco de Ibarra	N 24° 47′ 26.3″; W 104° 30′ 38.2”	Ancho y Tornachile
23	S. Juan del Río	Santa Rosalía	N 24° 53′ 44.6″; W 104° 26′ 17.4”	Ancho y Cola de Rata
24	S. Juan del Río	José María Patoni	N 24° 52′ 51.7″; W 104° 26′ 17.1”	Ancho

Sample number	Municipality	Plot	Geographic localization	Cultivar local name
25	S. Pedro del Gallo	La Laborcita	N 25° 29′ 21.3″; W 104° 24′ 03.0”	Ancho y Guajillo
26	S. Luis del Cordero	San Luis del Cordero	N 25° 24′ 42.0″; W 104° 16′ 03.0”	Árbol

Table 3.
Geographic localization of plots included in chili sampling implemented in plants showing plant wilting (CPW) symptoms at different municipalities of the state of Durango, México.

Sample number	Municipality	Plot	Geographic localization	Cultivar local name
1	Durango	CEVAG 1	N 24° 44′ 43″; W 103° 10′ 29.0”	Ancho
2	Durango	CEVAG 2	N 24° 44′ 43″; W 103° 10′ 29.0”	Ancho
3	Durango	CEVAG 3	N 24° 44′ 43″; W 103° 10′ 29.0”	Ancho
4	G. Victoria	J. G. Rodríguez	N 25° 26′ 45.2″; W 103° 44′ 57.3”	Guajillo
5	G. Victoria	A. Amaro	N 26° 32′ 36.6″; W 104° 08′ 15.8”	Jalapeño
6	G. Victoria	I. Allende	N 25° 18′ 12.6″; W 103° 58′ 20.5”	Guajillo
7	G. Victoria	C. Contreras	N 25° 16′ 25.3″; W 103° 58′ 20.5”	Guajillo

Table 4.
Geographic localization of plots included in chili sampling implemented in plants showing plant wilting (CPW) symptoms at two municipalities of the state of Durango, México. 2020.

and tissue samples were taken in the root crown and embryonic root. Samples were transported in paper bags with an identification label, including municipality, location, geographic coordinates and crop cultivar, then were sun-dried and stored at room temperature until processing.

3.2 Morphological characterization

Two classes of fungi (Anamorphic and Oomycota) were isolated including three different genera morphologically differentiated (**Table 5**). The most abundant genus was *Fusarium*, followed by *Rhizoctonia* and in a lower extent the Omicete *Pythium*. *Fusarium* were detected in 100% of the samples collected in 8 locations at municipalities of Simón Bolívar (2), Lerdo (1), Rodeo (1), San Juan del Río (3) and San Pedro del Gallo (1). This plant pathogen was present in variable proportions (18–100%) of samples at 25 locations (96%). *Rhizoctonia* were detected in 100% of the samples at five locations, such as: Simón Bolívar (2), Nombre de Dios (1), Poanas (1) and San Luis del Cordero (1). This fungus was present in 23 (88.5%) of the total samples with presence levels ranging from 18 to 100% across locations. A low incidence level was observed for *Pythium* and absence was registered at 16 locations (61.5%), with presence levels from 20 to 70% of the samples with the highest value (70%) at the location of J. Agustín Castro.

Results of the isolation frequency showed that *Fusarium* was the fungus present in most of the samples of plant material showing CPW symptoms, regardless of the sample site of origin, except for San Luis del Cordero. *Fusarium* was the widespread and frequently pathogenic genus of plant fungus, followed by *Rhizoctonia*, while in most of the sampling sites absence of oomycetes (*Pythium*) was found, mainly at the

	Municipality	Plot	Frequency (%)		
			Fusarium **sp.**	*Rhizoctonia* **sp.**	*Pythium* **sp.**
1	Simón Bolívar	Simón Bolívar	100	100	44
2	Simón Bolívar	R. Flores Magón	100	100	45
3	Lerdo	El Refugio	100	0	0
4	Lerdo	21 de Marzo	60	30	20
5	Mapimí	Perimex	60	40	60
6	Nazas	Francisco Sarabia	40	30	60
7	Nazas	Agustín Melgar	80	50	30
8	Nazas	Lázaro Cárdenas	40	70	50
9	Nombre de Dios	Francisco Munguía	40	100	0
10	Peñón Blanco	J. Agustín Castro	80	60	70
11	Poanas	Dago 1	33	67	0
12	Poanas	Pozo 12	73	27	0
13	Poanas	UTP 1	50	70	0
14	Poanas	UTP 2	89	33	0
15	Poanas	UTP 3	63	88	0
16	Poanas	Pilares	18	100	0
17	Rodeo	El Parián	63	38	38
18	Rodeo	F. Primo de Verdad	100	33	0
19	Rodeo	La Cuesta	75	58	0
20	San Juan del Río	El Crucero 1	100	0	0
21	San Juan del Río	El Crucero 2	100	18	0
22	San Juan del Río	Francisco de Ibarra	100	0	0
23	San Juan del Río	Santa Rosalía	70	60	0
24	San Juan del Río	José María Patoni	50	50	0
25	San Pedro del Gallo	La Laborcita	100	63	38
26	San Luis del Cordero	San Luis del Cordero	0	100	0

Table 5.
Isolation frequency of pathogen fungi related to chili pepper plant wilting at different municipalities of Durango, México.

municipalities of the semi-desertic region (Lerdo, San Juan del Río and Rodeo), but also in the highland valleys of Poanas and Nombre de Dios. Absence of *Pythium* was related to the phenological stage of sampled chili pepper populations since this fungus effect is mainly observed in the early stages of crop development. Soilborne pathogen *Fusarium oxysporum* and *Rhizoctonia solani* are the most common diseases causing root-rot and plant wilt in chili pepper cropping fields [36, 40]. These fungi are also observed in common bean and cereals planted in Durango for fodder and food, where the contamination with *Fusarium* species is one of the major sources of mycotoxins [41].

In the municipalities belonging to 'La Laguna' region (Mapimí and Nazas), the frequency of isolation of *Fusarium* was like that observed in other sampling sites, however, that of *Rhizoctonia* decreased and *Pythium* presence increased

considerably compared to the municipalities of the highlands region (Poanas) and that of the semi-arid sampling sites (**Table 5**). The use of mulch influenced increments for *Pythium* presence at some locations such as J. Agustín Castro.

In the second study, *Fusarium* also showed the highest presence scores (50 to 90%) at "Valle del Guadiana" and "Los Llanos" regions (**Table 6**). Other fungi species related to the common bean cropping systems such as *Uromyce*s and *Pythium*, also showed high presence levels. Other cosmopolitan fungi species *Rhizopus* (Zygomycetes) registered a high presence in plant samples, due to its omnipresent nature as an air contaminant, fast growing rate, and versatility of growth conditions (temperature and relative humidity) [42].

The low presence of *Phytophthora* fungus was observed in most of sampling sites (85.7%), due to increased novel sowing areas for chili peppers opened under irrigation at the municipalities of Durango and Guadalupe Victoria. Increments in the chili pepper area were related to recurrent crop complete losses registered in the main producing area of Poanas, Villa Unión and Nombre de Dios. Potato plantations also influenced the presence of *Phytophthora* fungus in both studied municipalities. Other fungi species (*Erysiphe* and *Cercospora*) were found, causing mildew in several crops and leaf or pod spots in common beans. Several fungi genera were detected in cultivated soils of Durango, causing severe economic losses in horticultural and agricultural crops.

3.3 Morphological identification

Different fungi and oomycetes show adaptation under different growth media, temperature, and light quality, then producing consistent characteristics that can be used for morphological identification [43]. The colony morphology of the *Fusarium* fungi species of the six most frequent isolates obtained in Durango was determined in pure culture using three different culture standard media: PDA (Potato Dextrose Agar, Difco®), Corn Flour Agar (CA) [44], and SDA (Sabouraud Dextrose Agar, Difco®) also known as Spezieller Nährstoffarmer Agar or Special Low-Nutrient Agar (SNA). Several characteristics were evaluated in fungi colonies, such as: pigmentation (color and hue) of the surface on the front and back of the colony, texture of the colony surface (cottony, resupinate, velvety, powdery, crustaceous, soaked, embedded, yeast-like, sticky, homogeneous or heterogeneous, presence or absence of elevation), margin type of the colony (smooth, regular, irregular, restricted, diffuse), pattern (radiated, flower-shaped or arachnoid), formation of resistance structures (sporodochia), mycelial type and growth rate.

Municipality	**Plot**	*Fusarium*	*Uromyces*	*Rhizopus*	*Phytophtora*	*Pythium*	*Erysiphe*	*Cercospora*
Durango	1	60	20	70	10	0	0	0
	2	70	20	0	20	0	0	0
	3	90	0	50	0	0	0	0
Victoria	*J.G.R	80	30	60	10	10	10	0
	A. A.	70	20	30	20	20	20	10
	I. A.	70	10	40	30	0	0	0
	C. C.	50	0	40	20	0	10	0

**J. G. R. = José Guadalupe Rodríguez, A. A. = Antonio Amaro, I. A. = Ignacio Allende, C. C. Calixto Contreras.*

Table 6.
Isolation frequency of pathogen fungi related to chili pepper root rot at different municipalities of Durango, México.

3.4 Colony characterization of *Fusarium* sp.

Strain EV1r. The growth of *Fusarium* sp. EV1r in PDA medium developed a fast-growing colony (4 days) with beige color (**Figure 3A**). The texture of the colonial surface was cottony presenting a smooth and irregular margin. On the other hand, the development of the strain e EV1r in corn flour agar (CA) developed a fast-growing white colony with a radial pattern and regular margin with a powdery texture (**Figure 3B**). In addition, the EV1r strain was also grown in SNA medium showing rapid growth (5 days) with a white front and back color with the presence of mycelium (**Figure 3C**).

Strain H1Zra. The growth of *Fusarium* sp. strain H1Zra in PDA medium developed a fast-growing colony (5 days) showing white color on both sides (**Figure 4A**). The texture of the colonial surface was cottony with the presence of aerial mycelium and irregular margins. On the other hand, the development of the *Fusarium* H1Zra strain on corn flour agar (CA) developed a fast-growing colony with white color, radial pattern, and regular margin. A velvety texture with the absence of aerial mycelium (**Figure 4B**) was also observed. In SNA (Synthetic Nutrient-Poor Agar) medium, the H1Zra strain showed slow growth (8 days) with an opaque colony and little mycelial development (**Figure 4C**).

Strain H1Zrb. The growth of *Fusarium* sp. H1Zrb in PDA medium developed a fast-growing colony (4 days) of white color with a slight yellow color in the periphery of the colony (**Figure 5A**). The texture of the colonial surface was velvety with the presence of mycelium with a regular margin. The development of the H1Zrb strain on corn flour agar (CA) developed a fast-growing white colony with a radial pattern and regular margin with a velvety and powdery texture with the absence

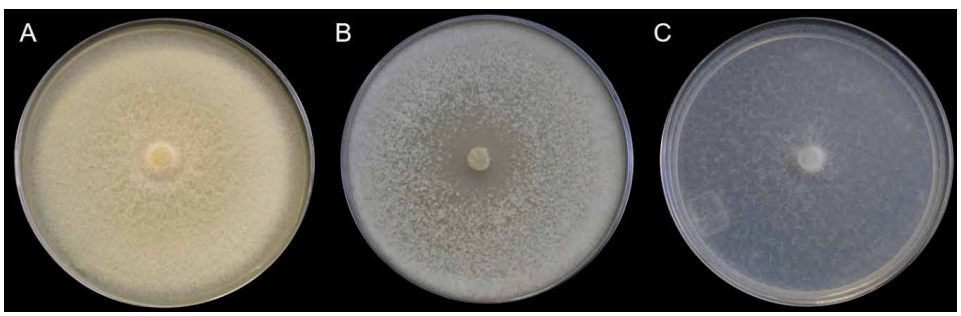

Figure 3.
Colony morphology study in Fusarium *sp. strain EV1r. Growth media: A) potato dextrose agar-PDA, B) corn flour agar-CA, and C) special low-nutrient agar-SNA.*

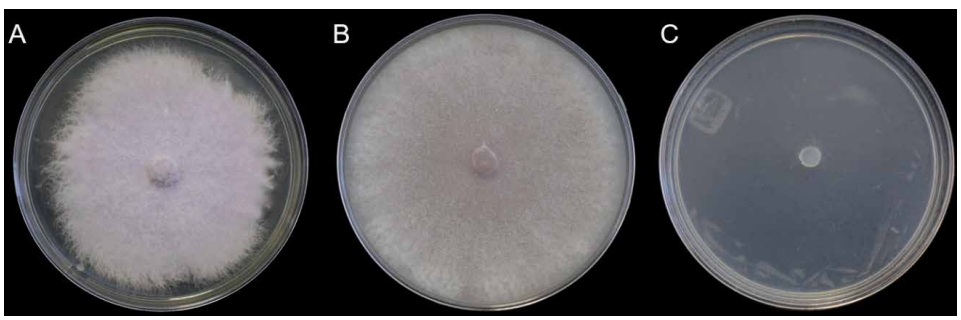

Figure 4.
Colony morphology study in Fusarium *HIZra strain. Growth media: A) PDA B) CA C) SNA.*

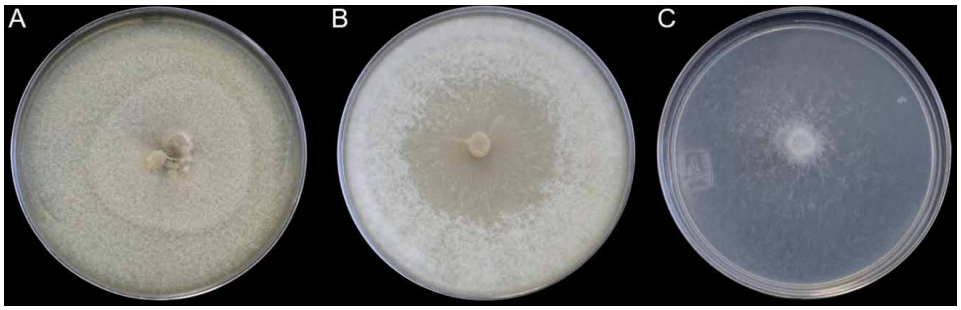

Figure 5.
Colony morphology study in Fusarium sp. strain HIZrb. Growth media: A) PDA B) CA C) SNA.

of aerial mycelium (**Figure 5B**). In SNA medium, the *Fusarium* sp. H1Zrb strain showed rapid growth with an opaque white colony with low mycelium development (**Figure 5C**).

Strain K4Zr. The growth of *Fusarium* sp. K4Zr in PDA medium developed a fast-growing white colony with the presence of growth rings (**Figure 6A**). The texture of the colonial surface is cottony with the presence of aerial mycelium with a regular margin. The development of the K4Zr strain on corn flour agar (CA) developed a rapidly growing white colony with a radial pattern and regular margin with a cottony texture with the presence of floccose mycelium (**Figure 6B**). On the other hand, in the SNA medium, the K4Zr strain showed slow-growth with an opaque white colony with a flat texture with little development of mycelium (**Figure 6C**).

Strain K5Zr. The *Fusarium* sp. strain K5Zr inoculated on PDA medium developed a slow-growth colony (8 days) showing opaque yellow color, with a floral pattern and irregular margin (**Figure 7A**). The texture of the colonial surface was creamy with the absence of aerial mycelium. The development of the K5Zr strain on corn flour agar (CA) developed a rapidly growing white colony with a radial pattern and regular margin with a cottony texture and abundant aerial mycelium (**Figure 7B**). In the SNA medium, the K5Zr strain showed restricted and diffuse growth with an opaque white colony and irregular margin (**Figure 7C**).

Strain C3WC. The growth of *Fusarium* sp. strain C3WC in PDA medium developed a slow-growing colony (4 days) of white color, with regular pattern and margins (**Figure 8A**). The texture of the colonial surface was flat and velvety with the absence of aerial mycelium. On the other hand, the development of the C3WC strain on corn flour agar (CA) developed a colony of white color with a slight brownish color of rapid growth. Radial pattern and regular margin with a cottony

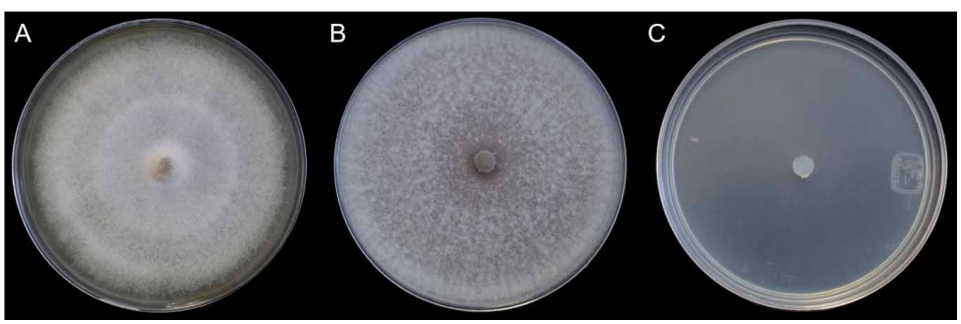

Figure 6.
Colony morphology study in Fusarium sp. strain K4Zr. Growth media: A) PDA B) CA C) SNA.

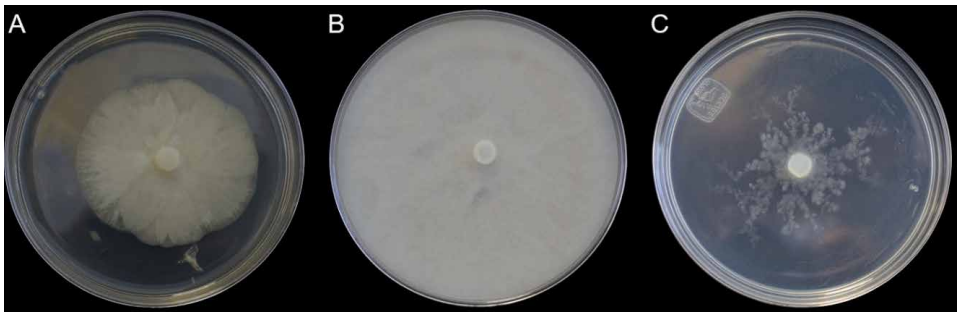

Figure 7.
Colony morphology study in Fusarium sp. strain K5Zr. Growth media: A) PDA B) CA C) SNA.

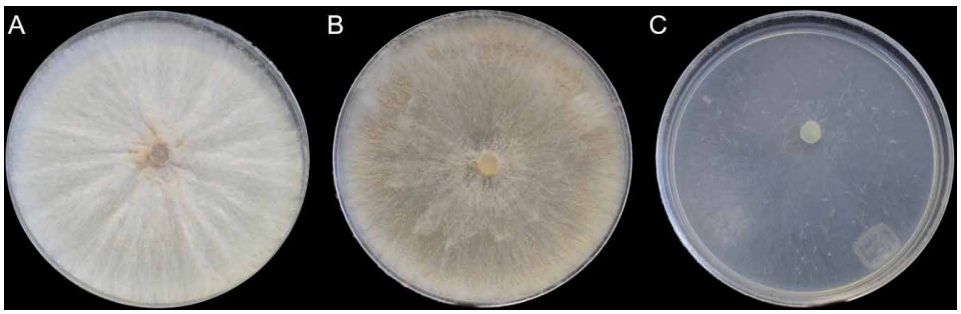

Figure 8.
Colony morphology study in Fusarium sp. strain C3WC. Growth media: A) PDA B) CA C) SNA.

texture and abundant aerial mycelium were also observed (**Figure 8B**). In the SNA medium, the C3WC strain showed a slow-growth colony with an opaque white color and irregular margins and little mycelium development (**Figure 8C**).

3.5 Genetic improvement

In México, 19 accessions of native chili pepper collected in the state of Morelos and 11 serrano chili accessions were selected considering its resistance to *Phytophthora capsici* that can be used in crop breeding [45, 46]. Twenty-six plant accessions were also identified in chili pepper with at least one individual showing resistance to *Fusarium* spp. and only two accessions from the gene bank resulted resistant to *P. capsici* and the mix including *Fusarium, Phythophtora* and *Rhizoctonia* [13]. Despite the germplasm selection for soil and seedborne fungal disease resistance no common bean and chile pepper cultivars for a specific response to soil fungi complex have been released in México.

3.6 Crop rotation

Crop rotation in the chili pepper production areas is influenced by farmer's tradition and the high economic income obtained with the fresh, dried and processed fruits, difficulting changes in the cropping systems. A similar response was observed for the common bean production under rainfed and irrigation monocropping systems. Then agronomic management recommendations need to be adjusted considering agroecological practices, including crop rotation, that contributes to improve the productivity and sustainability of local agroecosystems.

3.7 Agroecological practices

In common bean, chili pepper, maize and most of the crops, agroecological practices and integrated management systems need to be implemented and systematized. Agroecological practices are poorly used in current agriculture in North-Central México, and some components need to be validated at the commercial level including biofertilizers, organic matter incorporation into the soil and the use of natural pesticides, as well as crop choice and crop rotation. Other options include intercropping, relay intercropping, agroforestry with timber, fruit or nut trees; allelopathic plants use (sunflower), direct seeding into living cover crops or mulch, reduced tillage, drip irrigation, biological pest control, and cultivar choice [47].

In Durango, marginal advances were achieved in organic fertilizer production and use, most of the products are not often available in sufficient quantity. Industrial production has been obtained only for compost in "La Laguna" region but high prices have been observed making it unaffordable for farmers. Lombri-compost, fulvic acid and other liquid biofertilizers were also produced at low amounts and short time period effects, variable composition, and ambiguous results have been reported. Some interesting results were obtained by using Biological Nitrogen Fixing bacteria (*Rhizobium* spp. and *Azotobacter* spp.) and mycorrhizic fungi, but their production and distribution need to be reinforced. High biomass producing species with appropriate carbon to nitrogen ratio (25) has been selected (*Pennisetum* sp.) to reduce costs for direct organic matter incorporation into soils [48], stabilize pH (6.5–7.0) and naturally release soil minerals for plant nutrition.

Studies on organic pesticides need to be strengthened obtain clear results and to generate recommendations for commercial plantings of common bean, chili pepper and other food and fodder crops. Crop choices have been explored in Durango, using canola (*Brassica* spp.), chickpea (*Cicer ariethinum*), amaranth (*Amaranthus* spp.), barley (*Hordeum vulgare*), sunflower (*Helianthus annuus*), sorghum (*Sorghum bicolor*), and Oats (*Avena sativa*). Some problems need to be solved to improve adoption programs for these crops, such as mechanization from sown to harvest, efficient production storage and commercialization process.

Agroforestry with timber (Scott's Pine: *Pinus greggii*) was implemented using governmental programs, but then abandoned due to prolonged technical periods (10 to 12 years), poor technical support, and food requirement by farmer's families. Studies corroborated that the common bean is better adapted in early years of Scott's pine plantations compared to forage crops (oats and maize) [49]. Increments in Wichita pecan tree (*Carya illinoensis*) plantation area have been observed in the last 5 years in Durango, due to high prices of the pecan nuts and commercial competence to apple production, although influence on local agriculture has not yet been determined. Sunflower is an allelopathic crop which has been used in Durango to reduce problems observed with perennial grasses, mainly bermuda grass (*Cynodon dactylon*). Although, difficulties has been also observed for seed supply and during the crop harvest and seed (achene) commercialization processes.

Direct seeding into living cover crops was probed without success due to pressure exerted by beef/dairy livestock production and the preferential use of crop residues and cover crops as fodder instead as a natural amendment, then low organic matter content is commonly registered in agricultural soils. Low organic matter combined with alkaline reaction in the soils reduces the availability of nutrients, presence of beneficial microorganisms, water infiltration and retention, and then aggravating drought, plant nutrient deficiencies and disease problems in several food and cash crops. Mulch and drip irrigation is used in some cash crops, even common beans, in reduced areas due to installation costs, expensive maintenance and impractical use with actual machines used along the growth period

(sowing, mechanical weeding, cutting and threshing process). Reduced tillage also has been implemented in small areas of Durango, but then abandoned due to specialized machinery requirements, increment in weed populations and excessive herbicide use; as well as long periods required before registering yield increments.

The use of the biological pests control has been implemented releasing natural predators of plague-insects causing production problems, but no clear results were observed. Cultivar choice is present in common bean including several landraces (Negro San Luis, Bayo Rata, Canario), improved cultivars (Pinto Saltillo, Pinto Centauro, PID 1 and NOD 1) and breeding lines (PT14053, NGO14013), although low genetic variation has been observed for resistance to *Fusarium* and other fungi included in the root-rot complex. However, differences have been observed for plant surviving or escape strategies avoiding severe problems caused by root and aerial plant diseases.

In chili peppers difficulties have been observed for cultivar change, due to traditional use of specific open-pollinated cultivars (landraces), high seed prices for commercial hybrids and specific traits observed in landraces for fresh and dried fruits, as well as for processed fruit (chile pasado) flavor. Similar traits were considered in other chili pepper cultivars (puya, güerito) used for specific preparations included in Durango's cousine (chile con queso and frijoles charros). However outstanding results have been observed by using tomato 'big plant', produced under nursery conditions [50], and technology could be used for producing pathogen-free chili seedlings.

4. Conclusions

The *Fusarium* genus causes significant reductions in yield and seed or fruits quality in common bean, chili pepper and other crops sown in North-Central México. Low income for farmers and total crop losses are also observed in *Fusarium* infested plots affecting food availability and the local economy. Modern agricultural practices should be validated and implemented for sustainable production in common bean, chili and other important crops used in the Mexican highlands. Breeding for plant adaptation, disease resistance, water productivity and product quality are the main concepts in modern and sustainable agriculture.

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Fusarium is a large cosmopolitan genus of ascomycete fungi that are among the most important toxigenic plant pathogens causing seed and soil-borne diseases in a wide variety of agricultural crops worldwide. *Fusarium* species are broadly distributed in soil, root and plant tissues, and other organic substrates. Almost all species are able to generate mycotoxins, as secondary metabolites, that cause different physiological responses in plants. This book provides an overview of recent research on *Fusarium* species in the fields of metabolites, pathogenicity, plant-pathogen interactions, and management strategies in agricultural practices.

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