Fusarium verticillioides, a Globally Important Pathogen of Agriculture and Livestock: A Review

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Abstract

Fusarium verticillioides is a multi-phytopathogenic fungi widely distributed throughout the world in association with cereals and cereal based food products. Cereals are the basic staple food which provides much of the energy and protein for many populations, where 2534 MT consumed as food by Humans and animals. In some developing nations, grain in the form of rice, wheat or maize constitutes a majority of daily substance. In developed nations, cereal consumption is more moderate and varied as using cereal based products like corn flakes, oats, Poultry and animal feeds etc. Due to poor agricultural practices and intermittent rain at the time of harvest cereals are prone to contamination by number of fungi and it has become unavoidable and a worldwide problem. Fusarium species are the most common fungi associated with cereals all over the world. Among which F. verticillioides is the most frequently isolated species. FAO estimated that around 25-50% of cereals have been contaminated by mycotoxins. F. verticillioides produces secondary metabolites such as Fumonisins, trace level of fusaric acid, beauvericin, fusarin C, moniliformin, gibberiliformin in very low amount. Fumonisins receive the most attention as it is a potential carcinogen of global concern because they are the common contaminants of cereals and cereal-based foods. The International Agency for Research on Cancer (IARC) evaluated the toxin fumonisins as human carcinogenic.

ABBREVATIONS


INTRODUCTION

Fusarium verticillioides (Saccardo) Nirenberg (telomorph Gibberellamoniliformis Wineland) is an important plant pathogen with a wide range of hosts such as maize, sorghum, rice, millet, infecting plants in all stages of development, from the early hours of kernel germination to the time of harvest, including post-harvest deterioration of grains [1]. Seed infection by F. verticillioides is of major concern because it can reduce seed quality and result in contamination of grain with mycotoxins. Fusarium verticillioides infection of kernels occurs after flowering and is favored by hot and dry conditions. The fungus is distributed throughout the world, but predominant in humid tropical and subtropical regions and also present in the temperate regions [2,3]. In addition to causing plant diseases, infection by F. verticillioides can also result in contamination of kernels by fumonisins which can cause food safety problems for humans and animals and these fumonisins cannot easily be detoxified or removed from the grains [4,5].

TAXONOMY AND MORPHOLOGY

Fusarium verticillioides belongs to the section Liseola of Fusarium genus. In 1976, Helward Nirenberg rejected F. moniliforme and transferred Oospora verticillioides to F. verticillioides (Sacc.) Nirenberg, while retaining Saccardo as the original author, and the epithet “verticillioides” which described the whorled nature (i.e., verticillate or cyclic) of the conidiophores [6] and it has been defined as mating population A of the Fusarium fujikuroi species complex (formally known as Gibberella fujikuroi species complex) [7].

The taxonomical relationship of Fusarium verticillioides as follows: Kingdom Fungi, Class Deuteromycetes, Order Moniliales, Family Tuberculariaeae and genus Fusarium. Name of the taxon was highly controversial among the taxonomists as F. moniliforme and F. verticillioides. Presently the name F. verticillioides has been generally accepted and been in practise in the routine days [7]. The name F. verticillioides should be used only for strains that have the G. moniliformis (Gibberella fujikuroi species complex) as follows: Kingdom Fungi, Class Deuteromycetes, Order Moniliales, Family Tuberculariaeae and genus Fusarium. Name of the taxon was highly controversial among the taxonomists as F. moniliforme and F. verticillioides. Presently the name F. verticillioides has been generally accepted and been in practise in the routine days [7]. The name F. verticillioides should be used only for strains that have the G. moniliformis (Gibberella fujikuroi species complex)
mating population) telomorph and not simply as a replacement for *F. moniliforme*(Synder and Hansen). *F. moniliforme* is now likely called as *F. thapsinum* from sorghum, *F. sacchari* from sugar cane, *F. mangiferae* from mango, and *F. fujikuroi* from rice [5].

*Fusarium verticillioides* produces initially white mycelia but later develops into violet pigments with age. Macroconidia are long, slender, straight, thin walled, apically curved and notched basally with 3 to 5 septate and difficult to find. Abundant Microconidia are oval in shape, 0 septate, long chains of microconidia arise from monopodial and occasionally produces pair of rabbit ear shape of spores are observed. Chlamydospores are absent, swollen cells in some isolated species will be mistaken as pseudochlamydospores [5].

*Fusarium verticillioides* is morphologically similar to *Fusarium thapsinum* which do not produce yellow pigment and *Fusarium proliferatum* which produce short chain of microconidacin differentiated by molecular markers, production of spores and pigments. *F. verticillioides* is very similar to *F. andyazibut does not form pseudochlamydospores. *F. verticillioides* is similar in some respects to *F. nygamai* which forms microconidia in short chains or false heads from monopodial, abundant macroconidia in sporodochia and chlamydospores in the aerial hyphae in older cultures [8].

**HOST AND DISTRIBUTION**

*Fusarium verticillioides* is widely distributed throughout the world and is particularly associated with Maize [9,10], rice [11-13], sugarcane [14], wheat [15], banana [16], asparagus[17,18] and sorghum [19]. A total of 51 cereal samples were found to be associated with *F. Verticillioides* with 33.12% of percent incidence in maize in [21]. *F. verticillioides* were particularly associated with maize causes stalk rot and cob rot with drastic decrease of grain quality resulting in yield loss. The brutality of the rottness is affected by mode of inoculation systemically initiating from different routes such as seed or kernel through wounds in plant or infections of silks reciting disease symptoms [22,23].

The resistant genotypes are studied by the molecular mechanisms of the host response to infection which have been recently elucidated in maize and the identification of resistant genotypes will contribute to reduce fumonisin contamination. Developing Genetic resistance in maize to *F. Verticillioides* is of high priority in which sources of resistance has been identified and incorporated into public and private breeding programs [3]. Lanubile et al. [24], reported transcriptional changes were studied by next-generation RNA-sequencing for the first time with *F. verticillioides* infection causing diseases in the crop. Ramana et al. [52], demonstrated that genes Fum1 (Fum5), Fum6, and Fum8 were only present in *F. verticillioides* and other *Fusarium* species as the principle producers of fumonisins within the *G. fujikuroi* complex. Sanchez-Rangel et al. [51], reported similar results with a different pair of primers with presence or absence of the Fum1 gene which is the principle ability of a *F. verticillioides* isolate to produce fumonisins. Ramana et al. [52], system was based on the Fum1 and Fum13 gene sequences of *F. proliferatum* and *F. verticillioides* and was applied to the detection of the fungi in artificially contaminated cornmeal in a multiplex PCR assay.

**PHYSIOLOGY AND BIOCHEMISTRY**

*F. verticillioides* growth is reported to occur at 25°C and an osmotic potential of -1.0 MPa with the best growth occurring on wounded immature reproductive tissues. Fumonisin B1 production will also be high at this condition in the laboratory [31-33]. Biochemically many number of enzymes from *F. verticillioides* have been examined like ß-d-Galactosidase [34], Dextranase [35], D-lactonohydrolase [36], peptidase [37,38], peptidase [39], phosphatases [40], polygalacturonase [41-43], oxygenase [44], proteases [45], ribonucleases [46] and ß-xyllosidase [47]. *F. verticillioides* strains are commercially used to resolve DL-pantolactone mixtures since some strains can degrade lactic acid containing polymers [48,49]. Gonzalez-Jaen et al. [50], demonstrated that genes Fum1 (=Fum5), Fum6, and Fum8 were only present in *F. verticillioides* and other *Fusarium* species as the principle producers of fumonisins within the *G. fujikuroi* complex. Sanchez-Rangel et al. [51], reported similar results with a different pair of primers with presence or absence of the Fum1 gene which is the principle ability of a *F. verticillioides* isolate to produce fumonisins. Ramana et al. [52], system was based on the Fum1 and Fum13 gene sequences of *F. proliferatum* and *F. verticillioides* and was applied to the detection of the fungi in artificially contaminated cornmeal in a multiplex PCR assay.

**Table 1: Fusarium verticillioides infection causing diseases in the crop.**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Crop</th>
<th>Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coconut palm (Cocos nucifera)</td>
<td>Bud rot</td>
<td>Poetz R et al., 1999</td>
</tr>
<tr>
<td>2</td>
<td>Corn/Maize (Zea mays)</td>
<td>Fusarium ear rot, stalk rot, kernel rot, root rot, seed rot, Seedling blight, seedling root rot</td>
<td>Shurtleff M.C et al., 1993</td>
</tr>
<tr>
<td>3</td>
<td>Pearl millet (Pennisetum glaucum)</td>
<td>Top rot</td>
<td>Wilson LP et al., 1996</td>
</tr>
<tr>
<td>4</td>
<td>Sorghum (Sorghum bicolor)</td>
<td>Fusarium wilt head blight, root rot, stalk rot, Seedling blight and seed rot</td>
<td>Horne C.W et al, 1993</td>
</tr>
<tr>
<td>5</td>
<td>Sugarcane (Saccharum spp.)</td>
<td>Fusarium stem rot and twisted top</td>
<td>Ferreira S.A et al., 1993</td>
</tr>
<tr>
<td>6</td>
<td>Sunflower (Helianthus annuus)</td>
<td>Fusarium stalk rot</td>
<td>Gulya TJ et al., 1993</td>
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</tbody>
</table>

Source: www. apsnet.org/online/common/search.asp
Ma et al. [53], studied the genome statistics of *F. verticillioides* strain 7600 with NCBI accession number of AAIM02000000 and genome size of 41.7Mb comprising of 8 sequence coverage folds, 11 chromosomes, 31 scaffolds, 14,179 coding genes with 1,397bp median gene length and 0.36Mb repetitive sequence, 0.14% of transposable elements.

**SECONDARY METABOLITES**

Secondary metabolites are generally produced by all *Fusarium* species, but some mycotoxins are toxic to humans and animals. To date, 28 structurally related fumonisin analogues have been identified, only three of them fumonisin B1 (FB1), B2 and B3 occur abundantly, Fusaric acid and fusarin C is produced in very sensitive levels as of zinc and manganese occurs at fermentation time, trace levels of beauvericin, Gibberellin and moniliformin are produced not more than trace levels by *F. verticillioides* [54]. Among the *Fusarium* species *F. verticillioides* is the most prominent *Fusarium* species that produces the most important toxins fumonisins, discovered in the cultures of *F. moniliforme* (= *F. verticillioides*) [55,2].

**FUMONISINS AND ITS TYPES**

During the mid-1980s, although their effects on horses had been recognized for at least 150 years before with a significant risk of contamination to the association of *F. verticillioides* species with cereals and cereal based feeds [57,58]. During the last two and half decade, fumonisins have received worldwide attention. In 1988, the fumonisins were first isolated at the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC) of the Medical Research Council (MRC) in Tygerberg, South Africa, by Gelderblom et al [55]. Also in the same year, the structures of the fumonisins were also elucidated in a collaborative effort between the PROMEC and the Council for Scientific and Industrial Research (CSIR) in Pretoria [59]. Fumonisins are a group of 15 closely related mycotoxins that frequently occur in maize and other cereal based foods produced by 15 *Fusarium* species such as *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. thapsinum*, *F. anthophilum*, *F. globosum*, *F. fujikuroi*, *F. sacchari*, *F. nygami*, *F. dlamini*, *F. napiforme*, *F. andiyazi*, *F. pseudonygami*, *F. oxyssporum* and *F. Polyphialidicum* [56].

Fumonisins receive the most attention because they are the common contaminants of cereals and cereal-based foods. They are ubiquitous in distribution and are found frequently on freshly harvested and stored agricultural commodities such as cereals in all stages of their production, processing and storage. Fumonisins are divided into four groups: A, B, C and G, with the B-type fumonisins being the most toxic. There are more than 10 fumonisins, but only three, FB1, FB2 and FB3 occur naturally [60]. Fumonisin B1 is considered as the most serious threat to human and animal health and has been reported that FB1 makes up approximately 70%, and FB2 and FB3 each make up about 10–20% of the total fumonisin content [57,61]. The International Agency for Research on Cancer (IARC) evaluated the fumonisins as Group 2B carcinogens i.e. possibly carcinogenic to humans [62,63].

The chemical structure of fumonisins (Figure 1) was elucidated and named them as fumonisin B$_1$ (FB$_1$) and fumonisin B$_2$ (FB$_2$) respectively [55,59]. The fumonisin optimally produces at moderate water activity and with nitrogen limited and usually its production doubles roughly for every 48 hrs with increase in mycelial dry weight. Cultures of *F. moniliforme* MRC 826 on maize were used to isolate and to study the structure of the fumonisins. A few years later FB$_3$ and FB$_4$ were also isolated.

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<tr>
<th>Fumonisin</th>
<th>Formula</th>
<th>MW [g/mol]</th>
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<tr>
<td>FB$_1$</td>
<td>C$<em>{13}$H$</em>{35}$NO$_{15}$</td>
<td>721.8</td>
</tr>
<tr>
<td>FB$_2$</td>
<td>C$<em>{18}$H$</em>{39}$NO$_{14}$</td>
<td>705.8</td>
</tr>
<tr>
<td>FB$_3$</td>
<td>C$<em>{18}$H$</em>{39}$NO$_{14}$</td>
<td>705.8</td>
</tr>
<tr>
<td>FB$_4$</td>
<td>C$<em>{18}$H$</em>{39}$NO$_{13}$</td>
<td>689.8</td>
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*Figure 1* Morphological features of *Fusarium verticillioides*.  
A. Colony morphology, obverse; B. Micro-morphological features showing monophialides and long conidial chains.
and characterized [64,65]. Fumonisin B1 is a white hygroscopic powder that is soluble in water, acetonitrile-water or methanol and has the empirical formula C_{34}H_{59}NO_{15} (relative molecular mass: 721). Fumonisins B1 and B2 are stable in methanol if stored at −18°C but steadily degrade at 25°C and above. However, they are reported to be stable over a 6-month period at 25°C in acetonitrile-water (1:1). Fumonisin B1 is the diester of propane-1, 2, 3-tricarboxylic acid and 2S-amino-12S, 16R-dimethyl-3S, 5R, 10R, 14S, 15R-pentahydroxyicosane in which the C-14 and C-15 hydroxy groups are esterified with the terminal carboxy group of propane-1, 2, 3-tricarboxylic acid. FB2 to FB4 show different hydroxylation patterns.

**FUMONISIN DISTRIBUTION, METABOLISM AND ITS AFFECTS**

Fumonisins appear to be widespread in U.S. maize [66]. Surveys of 1,300 maize samples collected in the central United States from 1988 through 1995 indicated low levels of FB1 [67]. Cereals and cereal based products from maize source are the main commodities with natural FB1 occurrence have been reported from all parts of the world such as Brazil, Asia, Italy, Costa Rica and Hungary respectively [68-72]. In India, high levels of FB1 were reported in maize kernels infected with F. moniliforme [73, 74] and in maize as well as poultry feeds [75]. Fumonisin B1 contamination of maize and poultry feeds was high in Haryana, with 91 out of 100 maize samples and 42 out of 50 poultry feed samples were found to be contaminated with fumonisin B1. Fumonisins were considered as their occurrence was only confined to maize and later their presence was noted in a range of products, which include rice [76, 77], sorghum [75, 78, 79] and low levels in wheat, barley, soybean [80] and at very low level in beer [81, 82]. Recently fumonisin producing F. verticillioides was detected and differentiated from non-fumonisin producing strains through nested and multiplex PCR as an early detection method [83, 84].

The secondary metabolite fumonisins include the polyketide pigment bikaverin, the terpenoid plant growth regulators gibberellic acids (GAs), and multiple mycotoxins. Nelson et al. [31], reported that Fumonisin toxicity is thought to result from the blockage of sphingolipid biosynthesis [85]. Sphingolipids have a complex role in cell function by affecting a number of metabolic processes due to fumonisins. Accumulation of sphingolipid bases leads to inhibition of growth cells resulting in cytotoxicity. They can inhibit protein kinase-C, activate phospholipase D, activate or inhibit enzymes involved in lipid signalling pathways, inhibit Na+/K+ ATPase, and induce dephosphorylation of retinoblastoma a protein. All of these processes may increase cancer risk via loss of regulation of differentiation, apoptosis and lipid mediators that control cell proliferation [86-88]. Ceramide synthase inhibition generally results in accumulation of free sphinganine in liver, lung and kidney. Sphinganine, as a hydrophobic compound, can cross cell membranes and occur in blood and in urine if the kidneys are affected [89, 87]. As the proposed mechanisms of action involve alterations in de novo synthesis, nutritional factors might be important in toxic end-points. The liver is the target for FB1 in all animals and the kidney, is also a target in many animals. Initially fumonisin B induced toxicity is characterized by increase in apoptotic, oncotic necrosis and regeneration in kidney and bile duct hyperplasia is reported in liver. Fumonisin B1 toxicity depends on strain and sex of the rodents [31].

Marasas et al. [90], reported the first syndrome of fumonisins, ELEM, equine leukoencephalomalacia, in 1980s characterized by fatal necrotic lesions in the cerebrum in horses. Smith et al. [91], reported that fumonisins induce cardiovascular dysfunction in horses with decreased heart rates, lower cardiac output, and right ventricular contractibility which may be involved in the pathogenesis of the lesions in the central nervous system. The symptoms in swine have been referred to as Porcine Pulmonary Edema (PPE) characterized by pulmonary, cardiovascular and hepatic symptoms as a “mystery swine disease” with diarrhea, weight loss, increased liver weight and poor performance [92] (Table 2).

Toxicity of FB1 has been implicated affecting alligators [93], fresh water fish [94] causing hepatotoxicity in rats [95] with skin lesions [96], wounds [97], keratitis [98], Poly cystic kidney disease (PKD) mainly affecting liver, kidney and lungs in animals and life threatening cancer disease in humans [55] (Table 2). Subsequent studies have also shown that fumonisins are toxic to plants causing root rot, stem rot, seed rot, seedling blight, head blight diseases which has been explained in table one [77]. It is known to be allergic to humans systematically infecting cancer and HIV patients and not associated with hospital settings but nosocomial diseases do occur [99-102, 109]. F. verticillioides is resistant to most clinical antifungals like itraconazole, miconazole, amphotericin B [103] and flucytosine [104] reported as most effective.

**CONCLUSION**

Fusarium verticillioides is genetically the most intensively studied species in the section of *Fusarium*. This fungus is primarily a pathogen of maize and other crops like sorghum and largely responsible for important economic losses worldwide. *F. verticillioides* is mainly known to produce fumonisins which is well studied in terms of its synthesis, its effects on animals and humans that consumes contaminated grains its association

<table>
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<th>Table 2: Effects of fumonisins on humans and animals.</th>
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<td><strong>Affected</strong></td>
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<td>Horse</td>
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<td>Swine</td>
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<td>Chicken</td>
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<td>Primates</td>
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<td>Humans</td>
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with disruption of sphingolipid metabolism and folate transport which is a potential risk factor for human neural tubes. Hence it is a thrust area in food safety research for its prevention.

ACKNOWLEDGEMENT

We wish to thank Indian Council of Medical Research (ICMR) for awarding Senior Research Fellowship (SRF) as financial support to carry out the research work on Fusarium verticillioides and its management.

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