FUSARIUM SPECIES ASSOCIATED WITH MYCOTOXIN PRODUCTION CAUSED ANIMAL DISEASE IN NORTHWEST IRAN

SAREMI H* AND OKHOVVAT SM
Department of Plant Protection, College of Agriculture, University of Tehran, Karaj, Iran
*Corresponding Author: E Mail: hsn.saremi@gmail.com

ABSTRACT
The main crop witlings and animal diseases counting feed refusal, weight loss, death of cattle and sheep and chicken mortality were associated with Fusarium species in northwest Iran. Infected plants and feeds were collected from the fields and storages then cultured in Peptone PCNB Agar (PPA) as selective medium for Fusarium species after surface sterilization. Several Fusarium species were isolated from crop samples including potato tubers, wheat, corn, plant residues and animal feeds. Study showed most animal diseases especially chickens mortality were attributed to feeding infected plant straw and contaminated feeds in considered areas. The dominant recognized isolates were Fusarium solani, F. oxysporum, F. graminearum, F. moniliforme, F. sambutinum, F. culmorum, and F. equiseti as the main mycotoxin producers. The common Fusarium mycotoxins such as zearalenone, moniliformin and fusaric acid can be discovered from these species. The results put emphasis that Fusarium contamination of feeds or foods can be capable of the harmful consequences on animal and human health.

Keywords: Fusarium, Contaminated Feed, Mycotoxin

INTRODUCTION
Fusarium is one of the best characterized genera of soil fungi in terms of geographical distribution. Most Fusarium species occur in several continents and cause several diseases on various plants [1, 2]. Yield reduction in different crops is a major problem due to Fusarium-induced diseases in Iran. For example potato dry rot, damping off and decline of bean or pea, root rot and crown rot of wheat, bakanae disease of rice have been caused by Fusarium species resulted the extent of 40% or higher yield losses in the Zanjan Province. On the other hand, Fusarium includes several species that
produce mycotoxins associated with serious animal diseases, such as, feed refusal syndromes, estrogenic, fescue foot, degnal disease, moldy sweet potato toxicity, and bean hulls poisoning. Some of these mycotoxin-producing species have been also involved in human diseases including scabby grain intoxication, alimentary toxicity, and esophageal cancer [3-6].

Discovery of a growing number of naturally occurring Fusarium mycotoxins with serious threat to human and animal health caused worldwide interest in toxicogenic Fusarium species. Developments in mycotoxicology have also led to the discovery of secondary metabolites in Fusarium species [7-8]. These findings are based on weight loss and death of cattle and sheep in some parts of Iran. The cattle and sheep were occasionally fed with cereals or maize silo contaminated with Fusarium species, particularly in the Zanjan Province with a limited feeding season in this temperate zone.

The main agricultural crops, including potato, pea, bean, wheat, maize and rice, are common in northwest Iran and were found to be infected with various Fusarium species. The dry rot disease of potato in storage and planted tubers in the field is caused by Fusarium solani however; cultivars may differ in susceptibility to Fusarium pathogen. Some varieties are tolerant to infection when harvested but develop susceptibility during storage. Fusarium diseases occur in many parts of Iran but there were most common in the areas studied northwest Iran. It has been reported that Fusarium dry rot is a major problem in the potato growing areas all over the world [9-10].

The major legumes, such as, lentil, pea and bean also suffer from heavy yield losses due to Fusarium diseases including root rot, decline and damping off in the Zanjand Province. Root rot caused by Fusarium pathogen is detected in the reddish lesions on the taproot which later turn brown. The common root rot and decline or damping off of bean is spread in different countries where this crop is cultivated [11]. The infection causes not only yield reduction, it also produces mycotoxins like zearalenone ‘qv which is a major contaminant of maize and bean [12].

Scab or head blight disease of wheat caused by a Fusarium species occurs in some humid regions of the area under study. The disease is frequently severe in the wheat crop raised in a field saturated with residue from a previous host crop like wheat or corn. The infected crop produces immature heads in which one or more spikelets or the entire head appears prematurely dried. The grain from head-blighted fields is unsuitable for feeding farm animals as it may contain...
mycotoxins that induce muscle spasms and vomiting in humans and other no ruminant animals. The toxins apparently remain stable for years in the stored grain. Bread made from scabby wheat has been reported to be intoxicating [13]. Ingestion of contaminated grain and grazing on contaminated pastures has also established zearalenone to be the causal etiological agent in New Zealand [14]. The crown rot disease of wheat is also caused by another Fusarium species which results in white heads producing either shriveled or no grain. The disease could result in grain losses up to 40% or more in some locations of the Zanjan Province.

The Fusarium kernel or ear rot is also an important disease in all the areas studied, and white to pinkish mycelium can be seen in the severely infected plants. Extensive studies have dealt with various aspects of fumonisin mycotoxins, especially in relation to human risk assessment [15]. Occurrence of fumonisins in corn and their toxicity cause serious concern to animals and human health [16]. Disease of poultry in some parts of Iran may also be related to the consumption of feeds containing fumonisins. Some animal diseases and chicken mortality observed in the Zanjan Province were also reported from some other parts of the country. We have isolated many Fusarium species from plants, grain, animal feeds and human food that may affect human and animal health. This paper contains results of search to find causal agents of the major disease of potato, pea, bean, wheat and rice in northwest Iran. We have found that various Fusarium species can cause significant yield losses in these crops and their mycotoxins as secondary metabolites may be harmful to animals.

MATERIALS AND METHODS

Sample Collections

Samples of plants, grains, tubers and animal feeds suspected to be contaminated were collected from farms and stores located in northwest Iran, especially in the Zanjan Province. Tubers, roots, root crowns, stems and heads of potato, pea, bean, wheat, corn, and rice were cultured after surface sterilization with sodium hypochlorite to isolate Fusarium species. Since Fusarium species can also be isolated directly from soil, the dilution plate technique [17] was used by adding 1 g of soil from the vicinity of roots of infected plants to 0.05% water agar (WA) prepared by mixing 0.5 g agar in 1 litre water and used to prepare soil suspensions of different dilutions. One ml of soil suspension was uniformly spread over a selective medium such as PPA.

Wheat plants with symptoms of severe head blight disease were collected repeatedly from some humid areas, where problems
were encountered in animal feeding, to isolate the causal agent. Infected coleoptiles tissue and suspected seeds of wheat were cultured on common and selective media.

**Media and Identification**
All samples were transferred to the Fusarium Laboratory in Agriculture Faculty of the Zanjan University and were cultured on the common potato dextrose agar (PDA) and selective peptone PCNB agar (PPA), and carnation leaf agar (CLA) media after surface sterilization. The Fusarium colonies were sub cultured from PPA on CLA plates by transferring small mycelia plugs from the colony margins. The plates were incubated in an illuminated room under alternating temperature regime for two weeks to form sporodochia. All isolated cultures were examined after incubation and identified to species level using the methods described by Nelson et al., 1981 and Burgess et al., 1994 [18, 19].

**General Survey**
Information on animal diseases, chicken mortality, and contaminated feeds in different parts of Iran was received from the Department of Plant Protection, Faculty of Agriculture, Zanjan University.
The current situation of *Fusarium species* on crops and feeds in the country has been compared with the results of similar surveys in other countries to examine the possibility of mycotoxin production by *Fusarium* species in Iran.

**RESULTS AND DISCUSSION**
One of the main isolated species was *Fusarium solani* (Mart.) Appel & Wollen. Emend. Snyd. & Hans. as a cosmopolitan soil saprophyte and facultative parasite. This fungal pathogen caused root rots, stem cankers and storage rots of a variety of host plants in Iran. The fungus produced the sexual form called *Necteria haematococca* Berk. & Br. Which forms asci and ascospores in culture (*Figure 1a*).

Different *F. solani* strains were isolated from wheat, corn, bean, potato tubers, and other plants or foods and feeds consumed by man and animals. There have been serious cases of dry rot of potato caused by *F. solani* in the country [20], in particular, the Zanjan Province (*Figure 1b*). The Zanjan Province is one of the largest pockets of potato production in Iran.

*Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. was identified as a pathogen causing vascular wilt and damping off diseases in plants and isolated all over the country as a soil saprophyte (*Figure 2a*). This species produces several pathogenic strains that are specific to different hosts called forma specialis, e.g. *F. oxysporum* f. sp. *medicaginis* (Weimer) Snyd. & Hans. which infects alfalfa plant. This form was mostly isolated from alfalfa crop in the field.
and stored fodder in the Zanjan Province. *Fusarium oxysporum* f. sp. *pisi* caused root rot and damping off of pea with severe damage (Figure 2b) [21, 22].

Other mycotoxin producing species such as *F. sambucinum* Fuckel and *F. culmorum* (W.G. Smith) Sacc were also isolated from cereals, legumes, and potato tubers in the Zanjan Province, a temperate Mediterranean region. *F. oxysporum* was a dominant fungus in the crop fields, which caused root rot and stem rot in the bean crop (Figure 3). Legumes, especially bean, are cultivated over large areas in the Zanjan Province where many varieties are cultivated. *Fusarium culmorum* is a serious pathogen causing root, foot, stem and ear rots of cereals in Zanjan, and in a large number of other hosts all over the country. Death of chickens and loss of appetite or decrease in milk production of cattle were also reported from some regions. Plants and grains infected with *F. culmorum* can also accumulate high concentrations of mycotoxins, such as, deoxynivalenol, nivalenol and zearalenone [3].

*Fusarium graminearum* Schwab was the main causal organism of crown rot and head blight (scab) of cereals in the wheat belt of Iran (Figure 4a). The fungus was frequently isolated from the crown rot affected heads of wheat in different countries [23]. The incidence of head blight disease caused by *F. graminearum* was associated with rainfall in the temperate wheat growing areas. According to reports, a few sheep and cattle showed less appetite and other abnormal behavior in the affected regions. The grain from head blighted fields could be less palatable to livestock and contain mycotoxins that induce vomiting in humans and other non ruminant animals [13]. The toxins apparently remain stable for years in stored grain, and the bread made from scabby wheat can be intoxicating. Three closely related species of *F. graminearum* producing deoxynivalenol, nivalenol and zearalenone in wheat in this area were reported by Miller *et al.*, 1991, [24]. It was shown that wheat cultivars resistant to *Fusarium* head blight accumulated one-tenth deoxynivalenol than the susceptible ones [25]. Since barley is also infected with *F. graminearum* in some humid areas, it could as well cause feed refusal in animals. Deoxynivalenol production by isolates of *F. graminearum* from barley and wheat has been reported from Japan [26, 27]. Another common *Fusarium* species isolated from corn and rice in the area under study is *F. moniliforme* Sheldon. In fact, this was the most common *Fusarium* species associated with human and animal diet. We isolated many *F. moniliforme* cultures from samples of human food and animal feeds, such as, rice and corn in the Zanjan Province.
(Figure 5a). Microconidia of this species were formed in a chain (Figure 5b). Sexual stage is called *Gibberrella moniliforme* (Sheld.) Syn. Our findings on *F. moniliforme* confirm the observations of [28] who reported that the extract of an isolate of this species from moldy corn was toxic to chick embryos. Similarly, [29] found that an isolate of *F. moniliforme* from sorghum grain in the United States caused the death of two out of four 1-day-old White Leghorn chicks. *Fusarium moniliforme* was mostly associated with moldy corn in the Zanjan Province, where it was isolated from corn and other cereal grains. Actually, it has been suspected to be involved in human and animal disease since long as chicks consuming contaminated moldy corn died. *Fusarium equiseti* (Corda) Sacc. sensu Gordon was usually isolated from dead and dying plant tissues, seeds, roots, root crowns, stems, fruits and soil. It is a cosmopolitan species and occurs on plant straw in different climates. The animal diseases, especially in cattle, that were reported from some areas may be attributed to the feeding on plant straw from farms where outbreak had occurred. The degnala disease has been reproduced in the cattle fed with rice straw contaminated by *F. equiseti* during winter [30].

*Fusarium equiseti* was also isolated as a saprophyte and pathogen from feed and food materials in all the areas studied (Figure 6a) on dead and dying plant tissues, seeds, roots, and stems [6]. *F. graminearum* and ascospores of the sexual stage, *Gibberella* sp., was found associated with fruit rots of a wide range of plants.

Animal diseases such as degnala of cattle and mortality of chicks have been reported to be caused due to the use of rice straw and feeds contaminated by *F. equiseti* in some countries [31-32]. This cosmopolitan fungus and many other *Fusarium* species (Figure 6b) are common in northwest Iran, especially in the Zanjan Province.

The incidence of disease and contamination of feeds or potato tubers in store by *Fusarium* species were consistently high in different parts of Iran. Recently, high incidence of root rot and crow rot of wheat caused by *F. graminearum* (Group 1), renamed *F. pseudograminearum*, has been widely noticed and the fungus was isolated in the Zanjan Province. Crown rot disease of rice caused by *F. moniliforme* has also been noticed in the Zanjan and Gillan Provinces recently. This may cause sporadic sickness in animals consuming cereals infected by *F. graminearum* as happened in some countries including Australia, Canada, Finland, France, Germany, Hungary, Italy, Japan, UK, USA and others [33]. Isolates of *F. graminearum* have been examined to establish the possibility of mycotoxin...
production as it is known to produce secondary metabolites in corn [34].
In general, studies have confirmed economical losses in plants and animals in Iran due to several *Fusarium* species. For example, *F. sambucium* caused damage to bean and pea crops by damping-off or root rot diseases in the Abhar and Khoramdareh areas of the Zanjan Province [20]. The patch disease of grasses and crops caused by *F. solani* and *F. culmorum* is responsible for losses in the pasture grasses contaminated with mycotoxins like zearalenone in the Zanjan Province [35]. Mortality of chickens is also a serious problem in some locations of Iran, especially in the Zanjan Province. We cultured the contaminated feeds on common media such as PDA and isolated fungal species that may be responsible for toxicity. Several *Fusarium* species, in particular *F. graminearum*, were isolated from feeds suspected to cause chicken mortality due to mycotoxin production [33].
Other important *Fusarium* mycotoxins, such as fusaric acid and moniliformin, were reported to be the most common products of several *Fusarium* species [36, 37]. Fusaric acid, deoxynivalenol, and zearalenone were extracted from different *Fusarium* cultures and major mycotoxins have been isolated from suspected toxic duck and ostrich feeds that are known to cause health problems in poultry [38]. We have also isolated a few *Fusarium* species from human food that remained in store for long and potato and corn products like chips and popcorn. Consumption of moldy maize and sorghum containing funonisin mycotoxin has been associated with the outbreaks of food-borne diseases [39].
Moniliformin production in food and feed products has been reported from *Fusarium moniliforme, F. nygamai, F. oxysporum, F. avenaceum, F. acuminatum* and *F. equiseti* world wide [4, 5, 40]. We have isolated *F. Moniliforme* from rice plants and animal feeds in the Zanjan Province many times. We have also noted moniliformin to cause myocardial necrosis in animals which is similar to a serious heart disease observed in some regions of China [41].
Wortmannin is an antifungal antibiotic, inhibiting the growth of *F. graminearum*. However, it caused hemorrhage of heart, stomach, intestine and urinary bladder in rats. Wortmannin was first isolated from *F. oxysporum* and *F. sambucinum* in Norway, but later also found in other *Fusarium* species obtained from New Zealand and Alaska [42]. The effects of wortmannin may not be as severe as encountered with other *Fusarium* toxins, such as, zearalenone or moniliformin. It however caused food refusal and decreased immunological response in chicks and ducks [16, 43].
Wortmannin production was maximal in the Fusarium cultures grown on rice. Mycotoxins are also produced by other fungi, besides Fusarium species, that contaminate animal feeds and human food [8, 44]. Our investigations indicated that most animal feeds and items of human food materials like lime juice, vinegar, and jams were contaminated by fungal genera, including *Alternaria*, *Aspergillus*, *Penicillium*, and frequently *Rhizopus*, which produce mycotoxins. We believe, the mycotoxins occurring in human food, especially junk food, could be secondary metabolites of fungal origin. Some unknown human disease in different parts of the country, and possibly also pellagra, may be associated with the diseases prevalent in corn. The possible cause of pellagra was a fungus in corn [45].

In conclusion, mycotoxin production by the agriculturally important Fusarium species is one major factor that has stimulated international research on this fascinating fungal genus. A wide range of toxic compounds is produced by Fusarium species but the profile of secondary metabolites produced by most Fusarium species is still unclear. It is necessary to discover the diverse nature of the mycotoxins produced by them. The process of biosynthesis of these compounds or the extent of contamination of the food chain is still not fully understood.

![Figure 1: (a) Macroconidia of *Fusarium solani* and Asci with Ascospores of *Necteria solani*; (b) Dry Rot Disease of Potato Caused by *Fusarium solani*](image)
Figure 2: (a): Macroconidia and Microconidia of *Fusarium oxysporum*; (b): Chickpea Plant and other Crops; (c) Infected with *F. oxysporum f. sp. pisi* in the Zanjan Province

Figure 3: (a): Macroconida of *F. sambucinum*, (b): Infected crop of Common Bean in Abhar (Zanjan Province); (c) Infected Plant of Common Bean in Abhar (Zanjan Province)

Figure 4: (a): Head Blight or Scab Disease of Wheat; (b): Macroconidium of the Causal Agent
Figure 5: (a): Microconidia of *F. Moniliforme* in Chain; (b) Contaminated Cobs of Corn

Figure 6: (a) Spores of *F. equiseti*; (b) Colonies of Different *Fusarium* Species Isolated from Infected Crops and Feeds

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