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# Fungi associated with harvested corn grains of Golestan province in Iran

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## ABSTRACT

*Corn is one of the major crops, which is cultivation in different regions of Iran. Corn grains during harvest and post-harvest infected by several fungi seed borne, including toxin-producer fungi which the fungi are major share in digestive disease such as cancer in humans and animals. To determine mycoflora corn grains harvested was sample from maize cultivated regions in Golestan province such as Gorgan, Kordkuy, and Bandar gaz, Gonbad, Minoodasht and Kalale. To isolate fungi from maize seeds was used freeze Blotter method. In study, means of incidences Fusarium spp. were the highest (35.2%), followed by Aspergillus spp. (2.9%), Penicillium spp. (1.1%), Rhizopus spp. (2.3%), Mucor spp. (1.4%), and Alternaria spp. (0.2%). Among Fusarium species studied (51.8% of the total isolations), F. Proliferatum(90.1, 42.6%) had the highest percentages of frequency and the highest incidence in Gorgan. 3.5% of all samples were infected by A. flavus species which its of frequency and incidence were 2 and 40.2 percent, respectively and the highest level of infection was belonged to Bandare gaz seeds studied. Penicillium Species were isolated from most samples investigated which the highest incidence (2%) was in seeds studied in Kalale. In this study, among fungi species isolated, F. Proliferatum and A. flavus were identified as important as fungi seed borne and toxin-producer fungi in corn seeds in Golestan province.*

**Keywords,** corn grains, toxin-producer fungi, *Fusarium spp., Aspergillus spp.*

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## INTRODUCTION

Cereals have crucial role in the food basket of world people. And after wheat, barley and rice, corn is one of the most prominent crops that used as human food directly and cattle and birds food source indirectly. In Iran, corn is one of the most important crops which are cultivation in different areas.Dried weather in time of filling the grain and rain at the end of

season will be accompanied by toxin producer fungi infection of seeds such as *Fusarium* species which will be increased if the level of seeds moisture reaches to 18 to 20 percent at least [18]. *Fusarium* species rapidly colonize the corn residues in soil and being established in corn seeds. *F.proliferatum* and *F.verticillioides* species will remain in the residues of corn which are probably the main source of infection. Prevalence of seed borne fungi and seed infection by toxin producer fungi species play prominent role in existence of mycotoxic contaminations. Some of fungi species which are related to corn mostly belong to *Fusarium* (*Fusarium spp.*) and *Aspergillus* (*Aspergillus spp.*) genus. There are many reports that indicate these fungi species produce dangerous mycotoxin which can be harmful for human health and animals [1]. However, considerable information about mycoflora seeds corn is available in some corn producer countries such as Argentina, South America, Canada and many other counties [10, 12, 16]. There is not enough information about related corn fungi species, geographic distribution and abundance of toxin producer species in Iran.

Recently study on corn farmland in Iran indicated that these crops are infected by dangerous mycotoxins such as Fumonisin, Aflatoxin and Ochratoxin A. Shephard et al. [24] showed for the first time that maize grain of Mazandaran province can be highly contaminated with Fumonisins B1, B2 and B3. Tibi and Esavi [28] and also Yazdanpanah et al. [31] have shown that collected corn from south of Caspian Sea were contaminated by Aflatoxin and Ochratoxin. Surveys have shown Aflatoxin and Ochratoxin have natural prevalence in bread and red wheat respectively.

Previous studies showed that *Fusarium verticillioides*, *F.proliferatum* and seed born fungi species are mostly distributed in many Mazandaran province farms. Studies have shown that the most fungi isolates collected from northern corn farm of Iran were belonged to *F. verticillioides* and *F.proliferatum* after *Aspergillus* species. Studies on the fungi species isolated from cereals in Iran, proves that this fungi species have ability to produce aflatoxin, monilinformin, fumonisin and trichothecen. So far, 11 types of fumonisins have been identified which more often fumonisin B1 and B2 have been found in contaminated corn [8]. Fumonisins are the main causes of liver and kidney toxicity in many studied animal species. Additionally brain inflammation (Leukoencephalomalacia), lung inflation and liver cancer are other effects of using fumonisin in Livestock and Poultry [11, 8]. Due to there is high levels fumonisins in fungi culture, the International Agency for Cancer Research (IACR), toxin of *F.moniliforme* has classified as Potential Human Carcinogen Group(type 2B carcinogen) [30].

High amount of fumonisin which are along with *F.verticillioides* and *F.proliferatum* in corn have been reported in Europe, China, central Africa and also South Africa, south of America and Italy [27, 24, 4). *F.proliferatum* and *F.verticillioides* are reported in northern, southern and western corn seeds of Iran and in addition, *F.proliferatum* species is known as an essential component of corn seed mycoflora in Iranian corn [3,9]. Furthermore, different species of *Fusarium* have been reported in Iranian maize and also the contaminated corn by Fumonisin has been reported in Mazandaran and Esfahan province [6,31]. Study on harvested corn seeds in Golestan province farms and storages indicated that the content of fumonisin B1 in 23% and 6.5% of samples was 2 and 3 folds more than global recommendation amount (2000 ng/g) [17]. Moreover, this experience showed that samples were infected by fumonisin in all stages of harvesting (before and post harvesting time), drying and storing. This current study is aimed to investigate mycoflora in harvested corn in

different regions of Golestan province and also determine of toxin producer fungi species which are harmful for human health and animals in this area.

## MATERIALS AND METHODS

### Maize sampling method

A total of 99 samples (1.5–3 kg) of maize grain harvested in 2009 were collected at 6 locations in the Golestan province, in the main maize producing areas (Gorgan, Kordkuy, Bandar gaz, Gonbad, Minoodasht and Kalale). All the samples were taken from freshly harvested lots before drying and were dried in the sun for two days directly after harvesting. To isolate fungi from maize seeds was used Freeze Blotter method (FB). This method, for isolation of mycoflora maize grains, surface of every single collected maize kernel was disinfected by 1% Sodium-hypochlorite for one minute and rinsed twice in sterile distilled water for 30 seconds. Four hundred maize kernels (four series and each series contained on hundred seeds). Per subsample, 25 kernels were planted in each plate based on FB method [14]. Fungi colonies obtained by these two methods counted and identified then the different fungi species were subculture to Potato Dextrose Agar (PDA) medium culture.

### Fungi identification,

*Aspergillus* fungi species were identified by reliable identification keys of Raper and Fennell [22], Pitt and Hocking [21]. *Fusarium* isolates were purified by single spore method and then cultured on medium culture which was included Carnation Leaf Agar (CLA), SNA (Spezieller Nahrstoffarmer Agar) and PDA in 25 ℃ in 12 hours light and 12 hours darkness condition for 7 to 14 days. *Fusarium* isolates were identified by both morphology and characteristic of colonies based on Nelson et al [20] descriptions. Frequency, Relative Density and also incidence of fungi genera and species were counted by the following formulas [19],

$$Fr\ (\%) = (ns/N) \times 100$$
$$RD\ (\%) = (ni/Ni) \times 100$$
$$In\ (\%) = (ng/Ng) \times 100$$

Which Fr is frequency , ns is the number of fungi genera or species in samples, N is the number of samples, RD is Relative Density , ni is the number of isolated fungi genera or species and Ni is the number of all fungi, In, incidence, ng, number of infected grain, Ng, total number of grain.

### Statistical analyses,

Relative Density analyses and determination of differences between frequencies of fungi genera were done by Chi Square test, Also a data statistical analysis was performed by SAS (Statistical Analysis System) software.

## RESULTS AND DISCUSSION

### Fungi associated with maize grain

A total of 365 fungi isolates were recovered from 99 maize samples collected in the six main maize productions region in Golestan province during 2009. In this study, means of incidences *Fusarium spp.* were the highest (35.2%), followed by *Aspergillus spp.* (2.9%), *Penicillium spp.* (1.1%), *Rhizopus spp.* (2.3%), *Mucor spp.* (1.4%), and *Alternaria spp.*

(0.2%) (Table2). Among *Fusarium* species (51.8% of the total isolations), *F. Proliferatum*(90.1, 82.4%) had the highest percentages of frequency and relative density, also with the highest incidence in Gorgan (42.6%) and the lowest in Kalale(11.4%)(Table1). After *F. Proliferatum*, *F. verticillioides* was the most prevalent *Fusarium* species evaluated, with an incidence 18.4% in Gorgan (Table1). Based on the diversity and distribution of toxin producer fungi species, Gorgan had the largest proportion of infection toxin producer fungi species, especially *Fusarium* species (F. *proliferatum*, *F. verticillioides* and *F. moniliforme*) (Table1). In all six studied regions of Golestan province, *F. Proliferatum* (51.8, 88.5%) and *F. verticillioides* (44.1, 76.6%) had the highest frequency and relative density in comparison with other *Fusarium* species which total means incidences were 19.4 and 10.5 percent respectively (Table3). Because of fatal toxin which is dangerous for human and animals the prevalence of these fungi species in corn seeds is considerable. Some of these toxic metabolites that are produced by fungi such as fumonisin and T-2 toxin are the main reason of various cancers in digestive system. However, *F.verticillioides* species does not produce zearalenon and trichothecen but it is able to produce large quantities of fumonisin continuously while *F. proliferatum* can merely produce different amount of fumonisin [18]. *F.verticillioides* species has been reported from different parts of the world whereas *F. proliferatum* species is mostly found in temperate regions. Despite prevailing of corn ear rot disease among corn in Canada, low level of fumonisin infection has been reported [30]. Large amount of Fumonisin associated with *F. proliferatum* and *F.verticillioid es* have been reported from Europe, China, central Africa and also South Africa, south of America and Italy [13,18,25]. Additionally, the prevalence of *F. proliferatum* and *F.verticillioides* have been reported as important seed borne fungi and also toxin producers in corn from different parts of Iran and many other countries such as United States and Argentina [10,32 ]. The prevalence of *F. proliferatum* in Gorgan studied seeds was significantly higher than the other studied regions ($P< 0.05$, $P < 0.0001$) but there was not any significant difference between Gorgan and Kordkuy ($P> 0.05$). The prevalence of *F.moniliforme* (8.1%) and *F.verticillioides* (18.4%) was higher in Gorgan while the frequency of these two species were mostly in Kordkuy and Bandare gaz which were 46.5 and 86.4 percent respectively. However, there was not any significant difference between studied regions related to prevalence of *F.verticillioides* species ($P>0.05$). These results are accordance with other studies which have been done in Iran related to prevalence *of F.verticillioides* and *F.moniliforme* species. Additionally, Ghiyasian et al. [9] studied on fumonisin production ability of *F.verticillioides* and *F.moniliforme* in Khozestan, Mazandaran, Kermanshah and Fars in laboratory. They introduced both species as fumonisin producer while *F.verticillioides* produces the highest amount of fumonisin. Furthermore, natural corn infection by fumonisin producer species such as *F. proliferatum*, *F.verticillioides* and *F.moniliforme* has been reported from Esfahan and Mazandaran provinces, which this study found that Mazandaran samples had higher level of infection by fumonisin producer species in comparison with Esfahan samples [31]. High prevalence of *F.verticillioides* and *F.moniliform* species in Golestan confirmed to large amount of fumonisin B1in corn seeds in this province [17]. In this study, other *Fusarium* species that were present at low incidence levels were *F. culmorum*, *F. equiesti* and *F. acuminatum*(Table 1).

**Table1: Frequency, relative density and incidence of fungi species in maize from difference regions of Golestan provinces**

| Fungi isolated | Gorgan | | | Kordkuy Bandargaz | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **RD(%)** | **In(%)** | | **Fq(%)** | **q(%)** | **RD(%)** | **In(%)** | **RD(%)** | **In(%)** |
| *Fusarium proliferatum* | 90.1 | 82.4 | 42.6 | 89.4 | 50.9 | 12.2 | | 91.9 | 42.1 | 16.3 |
| *F.verticillioides* | 71.4 | 64.5 | 18.4 | 80.3 | 26.5 | 9.4 | | 86.4 | 53.2 | 8.5 |
| *F. moniliforme* | 36.2 | 14.2 | 8.1 | 46.5 | 17.2 | 3.1 | | 37.6 | 12.1 | 2.4 |
| *F. acuminatum* | 8.2 | 0.6 | 0.01 | nd | nd | nd | | nd | nd | nd |
| *F. eqiesti* | nd | nd | nd | 1.5 | 0.06 | 0.02 | | 2.6 | 0.03 | 0.02 |
| *F. culmorum* | 2.1 | 0.04 | 0.02 | 9.2 | 0.9 | 0.01 | | 7.6 | 0.5 | 0.2 |
| *flavus Aspergillus* | 48.2 | 4.2 | 2.6 | 50 | 2.8 | 1.9 | | 43 | 1 | 2 |
| *A. niger* | 29 | 0.6 | 1.5 | nd | nd | nd | | 31.3 | 6.2 | 3.4 |
| *Rhizopus pp.* | 42 | 0.2 | 1 | 29.2 | 6.3 | 1.8 | | 37.2 | 7.3 | 4.1 |
| *Mucor spp.* | 25 | 0.1 | 0.8 | 16.3 | 3.2 | 1.5 | | 18.4 | 4.2 | 2.1 |
| *Penicillium spp.* | 12.5 | 0.1 | 0.3 | 40.3 | 3.8 | 1.3 | | 38.9 | 1.6 | 0.9 |
| *Alternaria spp.* | 8.2 | 0.1 | 0.1 | 10.2 | 0.6 | 0.3 | | 13.5 | 0.7 | 0.4 |
| Total isolates | | 138 | | | 57 | | | | 52 | |
| Total samples | 37 | | | 15 | | | | 12 | | |

Fq = frequency; Rd = relative density; In = incidence; nd = not detected.

Table1: Continued.

| *Fungi isolated* | *Gonbad Kalale* | | | | | | *Minod* | | |
|---|---|---|---|---|---|---|---|---|---|
| **)** **RD(%)** | **In(%)** | | **Fq(%)** | **RD(%)** | **In(%)** | | **Fq(%)** | **RD(%)** | |
| | | | | | | | | | **In(%)** |
| *Fusarium proliferatum* | 86 | 49.2 | 21 | 85 | 37.2 | 12.9 | 89 | 49.3 | 11.4 |
| *F.verticillioides* | 70.3 | 39.6 | 10.3 | 79.2 | 42.1 | 8.1 | 72.3 | 39.1 | 8.4 |
| *F. moniliforme* | 41 | 14.5 | 4.5 | 39.1 | 21.6 | 3.9 | 40 | 12.5 | 2.1 |
| *F. acuminatum* | 7.3 | 0.09 | 0.01 | nd | nd | nd | nd | nd | nd |
| *F. eqiesti* | 1.9 | 0.05 | 0.02 | nd | nd | nd | nd | nd | nd |
| *F. culmorum* | nd | nd | nd | 12.1 | 0.2 | 0.9 | 10.6 | 0.1 | 0.5 |
| *flavus Aspergillus* | 40 | 6.2 | 1.9 | 24 | 2.1 | 1.3 | 36.2 | 4.9 | 2.3 |
| *A. niger* | 16.5 | 0.6 | 0.5 | nd | nd | nd | nd | nd | nd |
| *Rhizopus spp.* | 39.1 | 5.9 | 0.3 | 25.3 | 4.2 | 1.3 | 32.1 | 6.4 | 2.9 |
| *Mucor spp.* | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| *Penicillium spp.* | 27.2 | 1.2 | 0.6 | 35.2 | 2.1 | 1.5 | 31 | 2.9 | 2 |
| *Alternaria spp.* | 9 | 0.7 | 0.2 | 7.2 | 0.2 | 0.01 | 8.1 | 0.2 | 0.2 |
| Total isolates | | 37 | | | 48 | | | 33 | |
| Total samples | 10 | | | 15 | | | 10 | | |

Fq = frequency; Rd = relative density; In = incidence; nd = not detected.

**Table2 : Incidence percentage of fungi genera from from difference regions of Golestan**

| Regions sampled | *Fusarium* | *Aspegillus* | *Penicillium* | *Rhizopus* | *Mucor* | *Alternaria* |
|---|---|---|---|---|---|---|
| Gorgan | 69.1 | 3.5 | 0.3 | 1 | 0.8 | 0.1 |
| Kordkuy | 24.7 | 1.9 | 1.3 | 1.8 | 1.5 | 0.3 |
| Bandargaz | 27.4 | 6 | 0.9 | 4.1 | 2.1 | 0.4 |
| Gonbad | 35.8 | 2.4 | 0.6 | 3.1 | nd | 0.2 |
| Minoodasht | 25.8 | 1.3 | 1.5 | 1.3 | nd | 0.01 |
| Kalale | 22.4 | 2.3 | 2 | 2.9 | nd | 0.2 |
| Total average | 35.2 | 2.9 | 1.1 | 2.3 | 1.4 | 0.2 |

**Table3: Total means of Frequency, relative density and incidence of fungi species in maize**

| Fungi isolated | Total means | | |
|---|---|---|---|
| | Fq(%) | RD(%) | In(%) |
| *Fusarium proliferatum* | 88.5 | 51.8 | 19.4 |
| *F.verticillioides* | 76.6 | 44.1 | 10.5 |
| *F. moniliforme* | 40.07 | 15.3 | 40.01 |
| *F. acuminatum* | 7.7 | 0.3 | 0.01 |
| *F. eqiesti* | 2 | 0.04 | 0.02 |
| *F. culmorum* | 8.3 | 0.3 | 0.3 |
| *flavus Aspergillus* | 40.2 | 3.5 | 2 |
| *A. niger* | 25.6 | 2.4 | 1.8 |
| *Rhizopus spp.* | 34.1 | 5.05 | 11.7 |
| *Mucor spp.* | 19.9 | 2.5 | 1.4 |
| *Penicillium spp.* | 30.8 | 1.9 | 1.1 |
| *Alternaria spp.* | 9.4 | 0.4 | 1.2 |

Within the genus *Aspergillus*, *A. flavus* (48.2, 2.6%) was the most frequency and incidence in Gorgan samples studied (Table1). The highest mean incidence *Aspergillus* species (3.5%) was observed in Gorgan studied samples (Table2). From 3.5% of all samples were infected by *A. flavus* species which its frequency and incidence percentages were 2 and 40.2 respectively and the highest level of infection was also belonged to Bandare gaz seeds studied (Table1, Table3). There was not any significant difference between prevalence of this species in different regions of sampling (P>0.05). *A. flavus* has the highest ability to produce toxin in comparison to other *Asergillus* species and because of this high potential in toxin production such as Aflatoxin B1 and B2, this species is one of the most important fungus species related to human health and animals [5]. The frequency and incidence percentages of *A. niger* species was 25.6 and 1.8 respectively which the most infection was observed in Bandare gaz (2.6%) seeds studied (Table1). *A. niger* species is also considerable, because this species can produce dangerous toxin such as ochratoxin A [2]. Based on Marin et al. [15] reports, when three species of *A.flavus*, *A. niger* and *A. ochraceus* compete for an only food source at the same time, it may suppress the biosynthesis of fumonisin or degrade the produced toxin. In this study the co-occurrence incidence of *F. proliferatum* and *A.flavus* was 9.7 percent but any significant correlation was not observed between these two species (P >0.05). Zummo et al. [32] foud that simultaneous inoculation of *F. verticillioides* to corn ears can reduce the infection of *A.flavus*. In this study there was not any significant correlation between *F.verticillioides* and *A.flavus* species. This result accordace to the results of Ghiyasian et al. (2004) in Iran and Fennell et al. (1973) in United States. From 1.9% of all studied samples were contaminated by *Penicillium spp.* (Table3). The frequency and

incidence percentages of *Penicillium* species were 30.8 and 1.1 respectively, that the highest infection was observed in Kalaleh (Table1 and Table 3). There was not any significant difference between the prevalence of *Penicillium* species in different sampling regions (P> 0.05). Additionally, in this study any significant correlation was not observed between the prevalence of *F. Proliferatum, A. flavus* and *Penicillium spp*. species in different sampling regions (P> 0.5). Gonzalez et al. [10] found only 2.16% of maize grains to be contaminated by *Penicillium* species in Argentina while Ghiyasian et al. [9] made a study on four provinces of Iran and showed that the highest infection of *Penicillium spp.* (11.6) is belonged to Mazandaran province. Among the Zygomycetes, fungi of the genera *Rhizopus* and *Mucor* were found at an incidence levels was 11.7 and 1.4 percent respectively (Table3). Also Ghiyasian et al. [9] isolated these two species from majority of studied corn seeds in Fars, Khuzestan, and Kermanshah and Mazandaran provinces.

## CONCLUSION

Information about mycoflora in farms and also after harvesting (such as toxin producer fungi) the prediction and control of probable danger of their toxins will be possible. The infection seeds due to fungi and also their toxins are considerable as a threat of human health and animals and by detecting of this fungi feasible solution to reduce and omit their toxins and hazards' can be exploited. Based on these results corn seeds were mostly infected by toxin producer fungi such as *Fusarium spp*., *Aspergillus ssp*. and *Penicillium spp.* in Golestan while *F. proliferatum* and *A. flavus* played crucial role in infection of corn seeds and additionally, Gorgan had highest level of infected seeds in comparison with other studied regions.

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