

SYSTEMATIC INVESTIGATION AND FORECASTING OF MYCOTOXIN RISK  
IN TURKISH DRIED FIGS

by  
Ceren Uğurlu Akkaş

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SYSTEMATIC INVESTIGATION AND FORECASTING OF MYCOTOXIN RISK  
IN TURKISH DRIED FIGS

APPROVED BY:

Assoc. Prof. Dr. Özlem Güçlü Üstündağ .....  
(Thesis Supervisor)  
(Yeditepe University)

Prof. Dr. Fatma Yeşim Ekinici Kadıpaşaoğlu .....  
(Yeditepe University)

Prof. Dr. Mustafa Özilgen .....  
(Yeditepe University)

Assoc. Prof. Dr. Özlem Aytekin .....  
(Sağlık Bilimleri University)

Assist. Prof. Dr. Özge Seçmeler .....  
(Altınbaş University)

DATE OF APPROVAL: .... / .... / 20...

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Name, Last name Ceren Uğurlu Akkaş

Signature .....



*To my parents Kazim Uğurlu and Nuray Uğurlu*

*with all my love*

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## ABSTRACT

### SYSTEMATIC INVESTIGATION AND FORECASTING OF MYCOTOXIN RISK IN TURKISH DRIED FIGS

Fig (*Ficus Carica* L.) is a fruit of high nutritional value, with a significant food safety problem – mycotoxin, particularly aflatoxin (AF) contamination. Mycotoxin contamination in figs can be observed on the tree during pre-harvest and harvest, during processing, storage, and transportation. An in-depth investigation of the factors influencing mycotoxin contamination and predictive modeling studies are required for the development of effective control strategies and systems. In this thesis, The Rapid Alert System for Food and Feed (RASFF) database (2002-2020) was analyzed for mycotoxin hazard in Turkish dried figs. All the factors that can affect mycotoxin contamination (including weather conditions, agricultural and processing conditions, and non-product related factors (such as export quantities, regulatory changes)) were evaluated considering the yearly and monthly notification trends. Drought was identified as a key weather-related contributor to increased notifications in 2007 and 2012. Agriculture and process related factors that could have contributed to mycotoxin contamination in the investigated time frame were identified based on an in-depth review of academic and grey literature. A Recurrent Neural Network (RNN) based model was developed for the prediction of mycotoxin hazard (i.e. total number of RASFF notifications) from meteorological parameters for the primary dried fig producing regions in Turkey (İzmir and Aydın) (obtained from the Turkish General Directorate of Meteorology). The developed time series model predicted the total number of RASFF notifications with a  $r$  value of 0.64 and  $p < 0.05$ . A web-based analysis and forecast tool for AF risk in Turkish dried figs containing two modules was designed based on the developed RNN model: Module 1 (designed for public use based on total number of RASFF notification data) and Module 2 (designed for use by the Republic of Türkiye Ministry of Agriculture and Forestry based on mycotoxin contamination data from the Ministry database). This tool has great potential as a comprehensive system for the minimization of the mycotoxin risk in dried figs. Systematic collection and analysis of data on mycotoxin contamination routes and influencing factors throughout, from pre-harvest to transportation coupled with the forecasting capability would provide a strong foundation for control efforts aimed at minimizing mycotoxin risk.

## ÖZET

### TÜRK KURU İNCİRİNDE MİKOTOKSİN RİSKİNİN SİSTEMATİK OLARAK ARAŞTIRILMASI VE TAHMİNİ

İncir (*Ficus Carica* L.), besin değeri yüksek bir meyve olmakla birlikte mikotoksin, özellikle de aflatoksin (AF) kontaminasyonu nedeniyle önemli bir gıda güvenliği sorununa sahiptir. İncirlerde mikotoksin kontaminasyonu ağaçta, hasat öncesi ve hasat sırasında, işleme, depolama ve nakliye sırasında görülebilmektedir. Etkili kontrol stratejileri ve sistemlerinin geliştirilmesi için kontaminasyonu etkileyen faktörlerin derinlemesine araştırılması ve tahmine dayalı modelleme çalışmalarının yürütülmesi gerekmektedir. Bu tezde, Gıda ve Yem için Hızlı Uyarı Sistemi (RASFF) veri tabanı (2002-2020), Türk kuru incirindeki mikotoksin tehlikesi açısından analiz edildi. Mikotoksin kontaminasyonunu etkileyebilecek tüm faktörler (örn. hava koşulları, tarım ve işleme koşulları ve ürün bazlı olmayan diğer tüm faktörler (örn. ihracat miktarları, mevzuat değişiklikleri)), yıllık ve aylık bildirim trendleri dikkate alınarak değerlendirildi. Kuraklığın, 2007 ve 2012 yıllarında artan bildirimlerde hava durumuyla bağlantılı bir etken olduğu belirlendi. Araştırılan zaman çerçevesinde mikotoksin kontaminasyonuna katkıda bulunabilecek tarım ve prosesle ilgili faktörler, akademik ve gri literatürün derinlemesine incelenmesine dayanarak belirlendi. Mikotoksin tehlikesinin (toplam RASFF bildirim sayısı) Türkiye’deki başlıca kuru incir üretim bölgeleri için (İzmir ve Aydın) (Türkiye Meteoroloji Genel Müdürlüğü) meteorolojik parametreler kullanılarak tahmini için Tekrarlayan Sinir Ağı (RNN) tabanlı bir model geliştirildi. Geliştirilen zaman serisi modeli toplam RASFF bildirim sayısını  $r$  değeri 0.64 ve  $p < 0.05$  olarak tahmin etti. RNN modeli temel alınarak Türk kuru incirindeki AF riski için iki modül içeren web tabanlı bir analiz ve tahmin aracı tasarlandı: (toplam RASFF bildirimi sayısı dikkate alınarak herkesin kullanımına açık olarak tasarlanan) Modül 1 ve (Bakanlık veri tabanında bulunan mikotoksin kontaminasyon verileri dikkate alınarak Türkiye Cumhuriyeti Tarım ve Orman Bakanlığı tarafından kullanılmak üzere tasarlanan) Modül 2. Bu araç, kuru incirdeki mikotoksin riskinin en aza indirilmesine yönelik kapsamlı bir sistem olarak önemli bir potansiyele sahiptir. Mikotoksin kontaminasyon yolları ve hasat öncesinden başlanarak nakliye aşamasına kadar olan tüm süreç boyunca mikotoksin kontaminasyonu üzerinde etkili olabilecek faktörlerle ilgili verilerin sistematik olarak toplanması ve analizi, modelin tahmin yeteneği ile birleştiğinde, riski en aza indirmek için güçlü bir temel sağlayacaktır.

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## LIST OF SYMBOLS/ABBREVIATIONS

|       |                                          |
|-------|------------------------------------------|
| AF    | Aflatoxin                                |
| ARI   | Aflatoxin risk index                     |
| ARID  | Agricultural reference index for drought |
| BEA   | Beauvericin                              |
| BEN   | Balkan endemic nephropathy               |
| BGYF  | Bright greenish yellow fluorescence      |
| CPA   | Cyclopiazonic acid                       |
| DAS   | Diacetoxy-scirprenol                     |
| DON   | Deoxynivalenol                           |
| ENN   | Enniatin                                 |
| EFSA  | European Food Safety Authority           |
| FA    | Fusaric acid                             |
| RASFF | Rapid and Alert System for Food and Feed |
| RNN   | Recurrent neural network                 |
| OTA   | Ochratoxin A                             |
| ZEA   | Zearalenone                              |

## 1. INTRODUCTION

Aflatoxins (AFs) (B1, B2, G1, G2), the main mycotoxins in dried figs, are highly toxic dietary risk factors [1] and are classified as “carcinogenic compounds for humans” (group 1) [2]. AFs are the only mycotoxins for which maximum regulatory levels are established in the EU for dried figs for AFB1 at 6 µg/kg and for total AF (including B1, B2, G1, G2) at 10 µg/kg [3], while 8 µg/kg for AFB1, and 10 µg/kg total AFs are established in Turkey for dried fruits [4]. AF contamination of Turkish dried figs presents a serious food safety problem that impacts public health, trade and the economy. The dried figs that are exported from Turkey to the EU has been regularly reported due to mycotoxin limits (eg. 57 notifications about Turkish dried figs in 2020) in the Rapid Alert System for Food and Feed (RASFF) Portal [5].

There are different factors that can affect AF contamination of dried figs during pre-harvest, harvest, processing, storage, and transportation [6]. An in-depth investigation of these factors is required for the development of effective control strategies and predictive models. The online RASFF database, publicly accessible via the RASFF portal, is a valuable research resource that presents notification data for food safety hazards identified in food products sold in the EU market. RASFF notification data have been widely used in the literature for the investigation of food safety hazards in specific food products. Analysis of RASFF data for food products originating from Turkey has revealed mycotoxin contamination to be the most notified hazard category (i.e. 49% of all notifications) [7]. However, mycotoxin hazard in Turkish products, specifically in dried figs, has not yet been investigated. Therefore, in the first part of the thesis, a systematic investigation of the factors affecting mycotoxin contamination in Turkish dried figs, including product-related (such as weather conditions, and agricultural and processing conditions) and non-product-related factors (such as export quantities, regulatory changes) was carried out using monthly and yearly RASFF notification trends for the years 2002-2020.

Predictive modeling is a vital tool in managing and reducing mycotoxin risk in food products. Despite the well-documented correlation between meteorological factors (eg. drought conditions [8], rainfall, and humidity [9]) and AF risk in dried figs during the various stages of agricultural production and processing (a relationship supported by the findings of the first part of this thesis) this correlation has not been previously examined within

predictive modeling frameworks. Traditional approaches for the prediction of AF risk in different agricultural and food products have relied on empirical, mechanistic, and hybrid models [[10–14]. The availability of data, specifically for the quantification of AF risk, has been a significant consideration in these modeling studies. While the historical data required for predictive modeling may not always be available, generating the required data in the laboratory (for instance, for *A. flavus* infection [10–12]) can be rather time-consuming. The recent incorporation of machine learning methods into predictive modeling has offered some solutions, with neural networks standing out as one particular approach that can learn time dependencies and non-linear interactions from data [15,16]. Traditional methods, by contrast, have failed to take these factors into account, leading to limitations in prediction accuracy.

Therefore, in the second part of the thesis, predictive modeling of mycotoxin contamination of Turkish dried figs from meteorological parameters was carried out using the available RASFF data (i.e. total number of RASFF notifications) and meteorological data for the main fig producing regions in Turkey (Section 4). First, month-based and season-based models were developed for the prediction of the total number of RASFF notifications from meteorological data using multiple regression by Python. Then, recurrent neural networks (RNN) were used to develop a time-series model to account for time-dependencies and non-linearities in the data and to improve predictive performance.

While predictive models can provide the basis for the development of digital tools/systems that can support the decision-making of the actors involved in the control and minimization of AF risk before pre-harvest, the literature in this area is rather limited. There is a single study in the literature on the development of a tool for the prediction of AF risk from soil parameters based on the location and altitude of the fig orchards in the Southern Peloponnese [17]. However, the effect of meteorological factors on the prediction of contamination was not considered.

Therefore, in light of the results of Section 4, a web-based system was designed based on the developed RNN model for the monitoring and forecasting of AF risk in Turkish dried figs from meteorological parameters that the key actors in the sector can use. The risk monitoring and forecasting system included two modules: Module 1 (designed for public use based on the total number of RASFF notifications) and Module 2 (designed for use by

the Republic of Türkiye Ministry of Agriculture and Forestry based on mycotoxin contamination data from the Ministry database).



## 2. LITERATURE REVIEW

### 2.1. MYCOTOXINS AS FOOD SAFETY HAZARDS

#### 2.1.1. Mycotoxin: Types, Structures and Production

Mycotoxins are secondary metabolites produced by various fungi that can cause health problems in animals and humans due to their toxic effects. Mycotoxins are most commonly produced toxins by fungal species *Aspergillus*, *Penicillium* and *Fusarium* under appropriate environmental conditions (temperature and moisture). Some of the important mycotoxins for food safety are AFs that are produced by *Aspergillus*, ochratoxin A (OTA) that is produced by *Aspergillus* and *Penicillium*, fumonisins, zearalenone (ZEA), deoxynivalenol (DON), nivalenol, T-2 toxin, enniatins (ENN), and beauvericin (BEA) that are produced by *Fusarium*, patulin another mycotoxin which is produced by *Aspergillus*, *Penicillium* and *Byssoschlamys*, and ergot alkaloids produced by *Claviceps* [18–20]. Mycotoxin production by *Aspergillus* species has been the main food safety concern followed by *Fusarium* and *Penicillium* [21]. The chemical structure of selected mycotoxins are shown in Figure 2.1 [22].

One of the most important naturally occurring mycotoxins found in foods and feeds are AFs that are produced by various *Aspergillus* species. While 18 different types of AFs are known to exist, the most important ones are AFB1, AFB2, AFG1, and AFG2, which have different toxicity scale (AFB1>AFG1>AFB2>AFG2) [23]. AFs show fluorescence under UV light (363-365 nm), appearing B (blue, AFB1 and AFB2) and G (green, AFG1 and AFG2) [24].

The presence of fungi in the food product does not always indicate the presence of toxins as growth and toxin formation conditions are different. *A.flavus* and *A. parasiticus* are the main AF (AFB1 and B2; and AFB1, B2, G1, G2, respectively) producers in food samples [25]. *A. flavus* and *A. parasiticus* can grow in a wide range of temperatures from 12-42°C but the minimum water activity (aw) and pH range should be 0.80 and between 2-11, respectively, for the growth of most strains [26]. The optimum temperature of AF formation by *A. flavus* is 28-30°C, and by *A. parasiticus* is 25-35°C. The optimum pH, and aw values for toxin formation are 6, and 0.98, respectively [27]. Also, while at low temperatures equal amounts

of AF B and G are formed, at higher temperatures aflatoxin B production is higher than G production due to the catabolism of G [28].

OTA is another important mycotoxin, which shows possible carcinogenic properties for humans [29]. It is produced by *Aspergillus* and *Penicillium* species. *A.carbonarius* is a key species that can grow and produce OTA under optimum conditions of 15–25°C, and 0.95 aw, respectively [30].

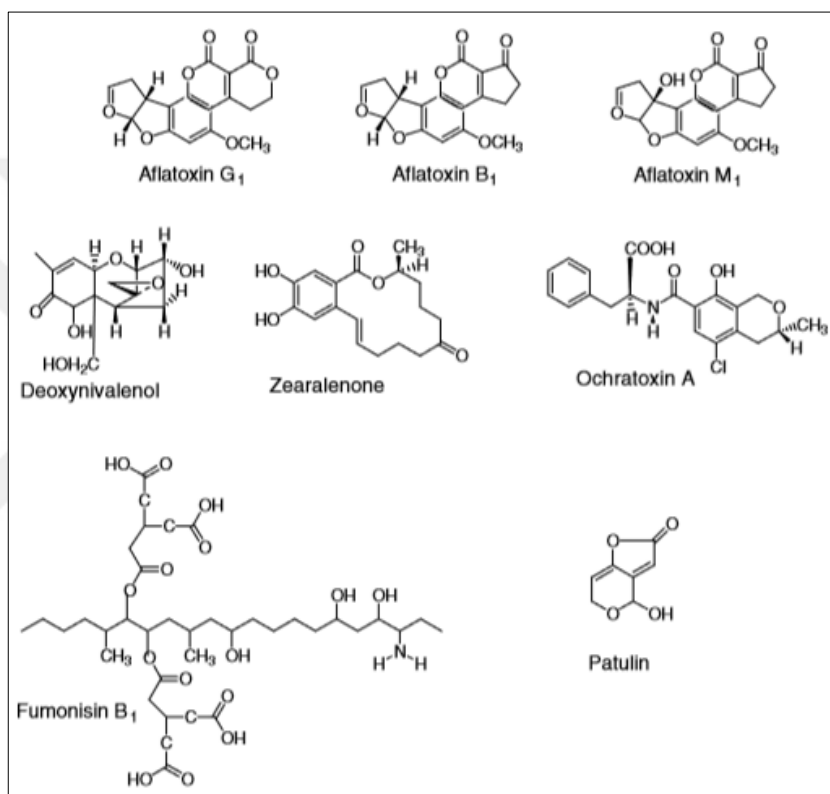


Figure 2. 1. Chemical structure of selected mycotoxins [22]

Fumonisin is another group of mycotoxins produced by *Fusarium* and *Alternaria* species. Among all fumonisins (B1, B2, B3), fumonisin B1 is the most common fumonisin (70%) [31]. It is produced by *Fusarium verticillioides* and associated with human esophageal cancer [32]. *Fusarium* is produced in warm and dry regions. *F. verticillioides* produces fumonisins at optimum temperatures of 20-30°C and *F. proliferatum* at 15°C with high aw such as >0.93 [33].

### 2.1.2. Occurrence and Health Effects

Mycotoxins are found widely in nature and occur in a wide range of agricultural and food products such as cereals, nuts, and fruits [34]. Therefore, they are important dietary risk factors for humans and animals that can be consumed chronically while dermal or inhalation exposure can be other routes of exposure. Mycotoxins present in foods and feeds may demonstrate carcinogenic, mutagenic properties or can cause many disorders like kidney disease in humans and animals [34]. Consumption of mycotoxins can also cause human fatality based on dose. Table 2.1 shows some human diseases caused by mycotoxins, the genera of fungi associated with them, and their food sources. AFBs, fumonisins, OTA, ergot alkaloids and ZEA are the mycotoxins with reported undesirable effects on human and animal health [34].

AFs are found in a wide range of foods and feeds such as cereals, rice, maize, peanut, and dried figs [35]. They are categorized as “carcinogenic for humans” (group 1) by the International Agency for Research on Cancer [2]. In 1974, 106 people died due to the ingestion of strongly AF contaminated maize containing high concentrations of AFB1 [36].

Table 2. 1. Effects of mycotoxins on human health

| Food                   | Fungi                                                         | Disease                  | Ref  |
|------------------------|---------------------------------------------------------------|--------------------------|------|
| Cereal grains, peanuts | <i>Aspergillus flavus</i> ,<br><i>Aspergillus parasiticus</i> | Hepatocellular carcinoma | [37] |
| Rice                   | <i>Penicillium citreonigrum</i>                               | Beriberi                 | [38] |
| Rye, cereal grains     | <i>Claviceps purpurea</i>                                     | Ergotism                 | [39] |
| Corn                   | <i>Fusarium verticillioides</i>                               | Esophageal cancer        | [40] |
| Grain, corn            | <i>Aspergillus ochraceus</i>                                  | BEN                      | [41] |

OTA is another important mycotoxin, which is classified as “possibly carcinogenic to humans” (group 2B) according to International Agency of Research on Cancer [2]. Main OTA producing species *A.ochraceus* and *A.carbonarius* are mainly found in dried foods, and grapes and wines, respectively. OTA causes Balkan Endemic Nephropathy (BEN) and urinary tract tumors in humans [42]. Fumonisin are another group of mycotoxins found

commonly in corn and corn products produced by *Fusarium* and *Alternaria* species and causes esophageal cancer [40]. Besides these, *Fusarium* species produce ENNs (A, A1, B, B1) and BEA that are found commonly in maize, grains and grain based products. While ENNs and BEA are found in grain based products, high content of BEA was also detected in dried fruits [43]. According to European Food Safety Authority (EFSA) (2014) [43], acute exposure of ENNs and BEA via grains and grain based products did not demonstrate any concern for human health while toxicity studies are needed for final judgement on health effects of chronic exposure in humans. Another toxin patulin is found mainly in apples followed by grapes, apricots, cereals and fruit juices (mainly apple). It is not carcinogenic while there are studies about its genotoxicity [44].

### **2.1.3. Stability of Mycotoxins During Food Processing**

In general, mycotoxins are known as stable compounds and cannot be destroyed during most food processing operations, which may lead to contamination of finished products. The mostly used processing steps that may affect mycotoxins are sorting, trimming, cleaning, milling, brewing, cooking, baking, frying, roasting, and canning. The stability of several mycotoxins during various methods of thermal processing has been reported in literature [45–49]. While sorting and trimming can decrease mycotoxin contamination via removal of mycotoxin contaminated material, both processing steps do not destroy the mycotoxins. Cleaning may reduce mycotoxin concentration in some food samples [50–52] but cannot completely remove all of the contamination. Mycotoxins may be distributed and concentrated in certain fractions during milling, yet they are not destroyed.

## **2.2. MYCOTOXIN HAZARD IN TURKISH DRIED FIGS**

Dried fig provides a suitable substrate for mycotoxin production of fungus due to its high sugar content (glucose, sucrose, and fructose (37.6%)) when compared with other fruits such as apricot, pineapple and raisins [53]. Mycotoxin hazard in dried figs presents an important public health and economic problem that needs to be controlled using effective strategies [54]. Contamination of mycotoxins in dried figs can occur on farm during pre-harvest and harvest, during processing, storage, and transportation.

While AFs (B1, B2, G1, G2) and OTA were the main mycotoxins reported in dried figs in the literature [8,55–66] presence of fumonisin B1, cyclopiazonic acid (CPA), patulin, ergosterol, diacetoxyscirpenol (DAS) [67–70], and more recently fusaric acid, ENNs, and BEA were also demonstrated [71,72]. In general, fumonisin contamination in figs occurs pre-harvest, while OTA and AF contaminations occur postharvest [6]. But, studies showed that contamination of AF and fumonisin started on the tree for dried figs [33,57].

### 2.2.1. Regulatory Mycotoxin Limits in Dried Figs

The Turkish Food Codex Regulation on Contaminants limits the AF levels (B1 and B1+B2+G1+G2) in dried fruits while other mycotoxins that can be found in dried figs (such as OTA, fumonisins, ENNs, BEA etc.) are not regulated [4]. Turkish Food Codex AF limits for dried fruits and EU AF limits for dried figs are presented in Table 2.2 [3,4].

Table 2. 2. Regulatory mycotoxin limits in dried figs

| Regulation                                        | Products                                                                                   | AF          | Max.<br>µg/kg |
|---------------------------------------------------|--------------------------------------------------------------------------------------------|-------------|---------------|
| Turkish Food Codex Regulation on Contaminants [4] | Dried Fruits<br><br>(Directly available for human consumption or used as a food component) | B1          | 8             |
|                                                   |                                                                                            | B1+B2+G1+G2 | 10            |
| European Commission Regulation [3]                | Dried Figs                                                                                 | B1          | 6             |
|                                                   |                                                                                            | B1+B2+G1+G2 | 10            |

Mycotoxin hazard in dried figs exported from Turkey has long been a concern in EU as EU inspection data, which is available publicly in the RASFF Portal [73], has consistently shown dried figs from Turkey to be the most notified product in the dried fruit category due to the presence of AFs [74], which led EU to establish extra control measures for Turkish dried

figs in 2009 increasing the control frequency at import from 10 to 20% [75]. While OTA was not regulated in dried figs, Germany set a limit for OTA in dried figs (8 µg/kg) in its national regulation in 2004 [76].

### **2.2.2. Aflatoxin Contamination in Turkish Dried Figs**

A review of the existing grey and scientific literature indicates mycotoxin – mainly AF-contamination to be a significant problem for dried figs produced in Turkey. A list of scientific studies on mycotoxin contamination of Turkish dried figs, including the samples analyzed, analytical techniques used and a summary of their results is provided in Table 2.3. All studies analyzed for AFs while the type analyzed varied – total, AFB1, and AFB2 [8,55–62,65,67,69,71,77–80]. There were limited studies detecting other mycotoxins in dried figs [57–60,62,67,69,71]. Total AF contents of dried figs were reported to be in the range of 0.05-1500 µg/kg (Turkish and EU regulatory limit: 10 µg/kg [4]) while OTA contents were 0.8-27 µg/kg (Table 2.3).

AF contamination has been a serious food safety concern in Turkish dried figs exported to EU as regularly reported by RASFF since 2010 [5,81–88]. AFs has been the most recurrent notification observed in Turkish dried figs (57 in 2020) [5]. The total AF content of final products sampled from exporter companies were in the range of 0.2-254.46 µg/kg [8,59,61,62,78].

Table 2. 3. Mycotoxin contamination of Turkish dried figs

| Sample                                                                                          | Crop/Product<br>Year | Mycotoxin                        | Mycotoxin contamination                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Ref  |
|-------------------------------------------------------------------------------------------------|----------------------|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Dried figs, from tree to final product<br>(from 11 orchards, 3 processing<br>facilities, n=103) | 1988                 | Total AF and<br>OTA              | AF contamination:<br>29% (AFB1: 0.5 - 63.0, AFB2: 0.5 - 37.7, AFG1: 0.5 - 78.3, AFG2: 0.5 - 12.5<br>µg/kg)<br>On the tree: 9/24, on the ground: 4/19, drying: 2/8, farmer store house: 1/3,<br>before processing: 7/12, during processing: 4/32, final product (fig paste): 3/5<br>OTA contamination: 3% (5.2-8.3 µg/kg)<br>No OTA contamination observed from tree, sun drying, store house and during<br>processing<br>On the ground: 1/19, before processing: 1/12, final product: 1/5 | [57] |
| Dried figs, during drying (n=3 kg)                                                              | 1993                 | Total AF and<br>OTA              | Drying (traditional harvest and sun dried): AFB1: 1/32 during drying (30 µg/kg)<br>OTA: 0/32                                                                                                                                                                                                                                                                                                                                                                                              | [58] |
| Dried figs (during drying, 7 facilities,<br>n=115)                                              | -                    | AF, CPA,<br>Fumonisin and<br>OTA | One or more mycotoxins: 94.7%<br>No contamination: 11.3%<br>Contamination: OTA: 47.8%, AF: 10%, FB1: 74.7%, CPA: 24.3%<br>Co-occurrence of OTA and FB1: 41%<br>Co-occurrence of AF and OTA: 4.3%                                                                                                                                                                                                                                                                                          | [68] |
| Dried figs, during processing<br>(palatable, fluorescent, cull; 4<br>processing facilities)     | 2004                 | AF, patulin                      | Palatable figs<br>Contamination: AFB1: 0.05 ppb, patulin: 4.8-25.5 ppb<br>Fluorescent figs<br>Contamination: Total AF: 117.9-471.9 ppb, patulin: 24.7-43.4 ppb                                                                                                                                                                                                                                                                                                                            | [67] |

|                                                                                              |                           |                   |                                                                                                                                                                                                   |      |
|----------------------------------------------------------------------------------------------|---------------------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
|                                                                                              |                           |                   | Cull figs<br>Contamination: Total AF: 0.8-8.3 ppb, patulin: 39.3-151.6 ppb                                                                                                                        |      |
| Dried figs, during storage, processing and drying (n=284)                                    | 1986                      | AFB1 and Total AF | Storage and processing: No AF contamination<br>Drying: AFB1: 112.3 ng/g; Total AF: 160.6 ng/g                                                                                                     | [56] |
| Dried figs, during processing (contaminated, after UV control) (n=50)                        | 2005                      | AFB1 and OTA      | Contamination: AFB1: 49/50 samples (0.7-222 ng/g)<br>OTA:32/50 samples                                                                                                                            | [60] |
| Dried figs, final product from Aydın food control lab. and bazaars<br>n=50                   | -                         | AF                | Contamination: 17/50<br>UV irradiation caused the degradation of AFs (25%)                                                                                                                        | [77] |
| Dried figs, final product, from <b>exporter companies</b> in Turkey<br>(n=1 kg)              | 2003, 2004                | AFB1 and OTA      | Only AFB1: 7/58; only OTA: 2/58; AFB1 & OTA: 2/58<br>(max AFB1: 35.1 ng/g, max OTA: 13.0 ng/g) in 2003<br>Only AFB1: 16/41; AFB1 & OTA: 2/41<br>(max AFB1: 20.6 ng/g, max OTA: 26.3 ng/g) in 2004 | [59] |
| Dried figs, final product, from <b>exporter companies</b> in Turkey<br>n=30 kg/0.5-10 tonnes | 2003, 2004,<br>2005, 2006 | AFB1, Total AF    | AFB1, 0.6, 2.0, 4.0 and 2.4% higher than 2 ng/g in 2003, 2004, 2005, and 2006, respectively<br>Total AF, 2.6, 3.0, 5.1, 2.7% higher than 4 ng/g (at the same time frame from 2003 to 2006)        | [78] |
| Dried figs, final product, <b>exporter companies</b> in Turkey<br>(n=4917)                   | 2007                      | AFB1, Total AF    | Contamination: 32% of dried figs (0.2–259.46 µg/kg), 9.8% of dried figs (higher than maximum limit, 2.04–259.46)<br>AFB1: 97%, AFG1: 47%, AFB2: 24%, AFG2: 6%                                     | [8]  |
| Dried figs, final product, <b>exporter companies</b> in Turkey<br>(n=264)                    | -                         | Total AF          | Contamination: Total AF: 313/2643 (0.2-162.76 µg/kg)                                                                                                                                              | [61] |

|                                                                        |                  |                                    |                                                                                                                                                                                                                                                                                                                                                                     |      |
|------------------------------------------------------------------------|------------------|------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Dried figs (sorted under UV light), <b>exporters</b> in Turkey<br>n=98 | -                | Total AF and OTA                   | Contamination: OTA: 18/98 (0.87-24.37 ng/g), Total AF: 7/98 (0.23-4.28 ng/g), OTA & Total AF: 2/98 (0.23-1.02 ng/g)                                                                                                                                                                                                                                                 | [62] |
| Dried figs, <b>retail</b> in Turkey<br>(10 cities, n=130)              | 2013, 2014, 2015 | AFB1, Total AF                     | Total AF: 16/130 (0.1-28.2 µg/kg), AFB1 (0.1-12.5 µg/kg)<br>Mean intake of AFB1 was 0.003 ng/kg bw/day<br>Mean intake of Total AF was 0.004-0.005 ng/kg bw/day                                                                                                                                                                                                      | [65] |
| Dried figs, final product, <b>retail</b> in Switzerland<br>(n=90 kg)   | 1985             | AFB1                               | AF: 62/2500, AFB1: 22.6 ppm (decreased to 0.3 ppm by removing BGYF figs)                                                                                                                                                                                                                                                                                            | [55] |
| Dried fig and fig paste, final product, <b>retail</b> in UK            | 1988, 1989       | Total AF**                         | Dried fig: Total AF: 8/93 (9% > 10 µg/kg)<br>Fig paste: Total AF: 12/112 (11% > 10 µg/kg)                                                                                                                                                                                                                                                                           | [79] |
| Dried figs, final product, <b>retail</b> in Brazil                     | 2002, 2003       | AFB1, AFB2                         | Contamination: AFB1&AFB2: 11/19<br>1 sample, 1500 µg/kg<br>10 sample, 0.3-2 µg/kg                                                                                                                                                                                                                                                                                   | [80] |
| Dried figs, final product, <b>retail</b> in Spain (n=28)               | 2012, 2013       | AF, OTA, DAS, Fumonisin, BEA, ENNs | Spanish Market<br>Contamination: BEA (822 µg/kg mean, 5.19-3620 µg/kg) and DAS (77.5 µg/kg mean)<br>Tunisian Market<br>Contamination: AFG1 (5 µg/kg mean, 3.96-6.38 µg/kg) and OTA (<LOQ)<br>Spanish & Tunisian Markets Contamination:<br>ENNB (132 µg/kg mean, LOQ-251 µg/kg)<br>ENNB1 (10.2 µg/kg mean, LOQ-34.8 µg/kg)<br>ENNA1 (4.1 µg/kg mean, LOQ-14.7 µg/kg) | [71] |

### 2.2.3. Dried Fig and Dried Fig Production

Fig (*Ficus Carica L.*) is a high nutritional value fruit that includes phenolic compounds, organic acids, carbohydrates, vitamins, minerals and fibers. It was first cultivated in the Mediterranean Region about 6000 years ago. Hundreds of wild and cultured species of figs have been reported while most grown varieties in the commercial fig industry differ according to regions. Figs are mainly produced in Turkey in the provinces of Aydın, İzmir, and Bursa. "Bursa Black" (fresh) and "Sarilop" (dried) are the main commercial varieties cultivated in Turkey followed by "Goklop", "Yediveren", "Morguz", "Sarı Zeybek" and "Ufak Yeşil" [89]. The quality of figs depends on fruit size, maturity period, fruit shape, neck length, fruit skin cracks, ease of peeling, ostiole width, total soluble solids and acidity according to their varieties and the regions which they are grown in [89,90].

The short shelf life of fresh figs has led to the usage and storage of figs in dried form in order to increase their shelf life. Dried fig has a wide range of intended use as dried snack fruit, as raw material for fig products such as fig doner/fig paste, fig delight, fig jam and fig pekmez, and as an ingredient in biscuits, candies and in the bakery sector. The description and specification of dried figs with intended uses is demonstrated in Table 2.4.

Table 2. 4. Description of dried fig product (adapted from FAO, 2001)

|                                                 |                                                                                                                                                                                      |
|-------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name of Product</b>                          | <i>Ficus Carica L.</i> , commonly as dried fig                                                                                                                                       |
| <b>Description</b>                              | Dried snack fruit                                                                                                                                                                    |
| <b>Customer specification</b>                   | Uncracked, shriveled, uncontaminated with fungi, not too dried fruits<br>AF limit 8 µg/kg B1, 10 µg/kg total AFs in Turkey [4]<br>AF limit, 6 µg/kg B1, 10 µg/kg total AFs in EU [3] |
| <b>Conditions of storage (finished product)</b> | Moisture content (<22%), cool and dry conditions                                                                                                                                     |
| <b>Shelf Life</b>                               | 6 months                                                                                                                                                                             |
| <b>Intended use</b>                             | Snack food, traditional foods (fig doner/fig paste, dried fig, fig jam, fig pekmez), diced dried figs                                                                                |

|                        |                                                   |
|------------------------|---------------------------------------------------|
| <b>Packaging</b>       | Differs product based (paper box, aluminium etc.) |
| <b>Target Consumer</b> | European, US and Turkish                          |

Turkey has an important position in fresh/dried fig production in the world as the largest fig producer and exporter followed by Egypt, Morocco, Algeria and Iran [91]. Thirty percent of the fig production in Turkey is consumed in the domestic market as fresh, while 70% of figs are consumed and exported as dried figs [92]. Dried fig production in Turkey increased from 55600 tons in 2004/2005 to 78200 tons in 2017/2018 season [92].

In general, dried figs can be exported as palatable (dried fig), fig paste, culls figs (not appropriate for direct human consumption due to the physical defects of dried figs, appropriate for industrial processing) and diced dried figs according to the Turkish Ministry of Customs and Trade General Directorate of Cooperatives Report [93] and provides high economic gain to Turkey (Table 2.5).

Table 2. 5. Dried figs and products export data in Turkey (2010-2016)

| Year    | Dried Fig |        | Fig Paste |        | Diced<br>Dried Fig |            | Cull and<br>Other Figs |        | Total |        |
|---------|-----------|--------|-----------|--------|--------------------|------------|------------------------|--------|-------|--------|
|         | Ton       | 1000\$ | Ton       | 1000\$ | Ton                | 1000<br>\$ | Ton                    | 1000\$ | Ton   | 1000\$ |
| 2010/11 | 36.26     | 136.17 | 6.54      | 9.86   | 464                | 894        | 2.42                   | 6.94   | 36.26 | 136.17 |
| 2011/12 | 33.86     | 122.87 | 3.69      | 5.72   | 488                | 892        | 2.18                   | 6.01   | 33.86 | 122.87 |
| 2012/13 | 40.85     | 142.79 | 7.27      | 10.15  | 325                | 102        | 2.87                   | 7.44   | 40.85 | 142.79 |
| 2013/14 | 50.58     | 183.74 | 8.78      | 14.57  | 940                | 2.56       | 3.86                   | 10.84  | 50.58 | 183.74 |
| 2014/15 | 55.64     | 243.26 | 8.41      | 15.09  | 642                | 1.29       | 4.15                   | 14.50  | 55.64 | 243.26 |
| 2015/16 | 41.30     | 166.35 | 9.82      | 14.75  | 263                | 693        | 4.40                   | 12.08  | 41.30 | 166.35 |
| 2016/17 | 41.14     | 173.23 | 7.31      | 10.85  | 399                | 749        | 4.51                   | 12.07  | 41.14 | 173.23 |

The processing steps of dried figs are demonstrated in Figure 2.2. The moisture content of figs on the tree (firm ripe figs) is 68-74%. The drying process of the figs starts on the tree and continues as they fall to the ground when the moisture content reaches approximately

25-37% (shriveled figs) [57]. Figs are collected by workers (from the ground) in order to apply sun-drying.

Drying step decreases the moisture content to safe levels that prevent growth of fungi and toxin production. In this step, it is important to provide homogeneous drying process for each fig. After sun-drying in plastic or wooden trays for approximately 5 days, samples are stored in farmer store-house. Then, dried figs come to the processing facility and raw material undergoes laboratory inspection before processing for physical defects, moisture content and AF contamination by experts. After that, fumigation is applied to the raw materials in order to prevent living insect pests. While methyl bromide is not approved in the EU [94] and there is a Turkish regulation to decrease its usage in agriculture due to its thinning effect on the ozone layer [95], methyl bromide is still used for fumigation of dried figs in Turkey as indicated in the Annual Dried Fig Report of the Turkish Ministry of Commerce General Directorate of Craftsmen and Cooperative (2019) [93]. After fumigation, pre-sorting is applied for the separation of sour, crack and contaminated dried figs (black moldy). Then, dried figs are sorted according to the classifications specified by Turkish Standard [96] as Extra, Class I, Class II and Industrial grades (Table 2.6) and size of dried figs (Table 2.7).

Table 2. 6. Dried fig classification specifications (TS 541)

| Defects                                                                   | The maximum % of defected dried figs based on 1000 g sample |         |          |                  |
|---------------------------------------------------------------------------|-------------------------------------------------------------|---------|----------|------------------|
|                                                                           | Extra                                                       | Class I | Class II | Industrial Grade |
| <b>Insect damage</b>                                                      | 9.0                                                         | 12.0    | 25.0     | Free             |
| <b>Sunburned, damaged, cracked, excessively dried and similar defects</b> | 8.0                                                         | 10.0    | 25.0     | Free             |
| <b>Moldy</b>                                                              | 3.0                                                         | 4.0     | 5.0      | Free             |
| <b>Foreign body</b>                                                       | 0.5                                                         | 0.5     | 0.5      | 2.0              |
| <b>Total tolerance</b>                                                    | 10.0                                                        | 15.0    | 30.0     | Free             |

Table 2. 7. Dried fig size specifications (TS 541)

| Size Number | Fruit number in 1 kg |
|-------------|----------------------|
| 1           | $\leq 40$            |
| 2           | 41-45                |
| 3           | 46-50                |
| 4           | 51-55                |
| 5           | 56-60                |
| 6           | 61-65                |
| 7           | 66-70                |
| 8           | 71-80                |
| 9           | 81-100               |
| 10          | 101-120              |
| 11          | $>121$               |

After the sorting steps, AF control is performed by manually removing the AF-contaminated dried figs that show bright greenish yellow fluorescence (BGYF) under (365 nm) UV light [55]. This step reduces AF levels significantly by eliminating AF contaminated dried figs and preventing potential AF contamination of other figs via contact [55]. While UV light sorting can reduce the AF level of dried figs significantly (for example a reported decrease in AFB1 level from 22.6 ppb to 0.3 ppb) [55] elimination of all AF contaminated samples is not attainable due to the potential growth of fungi inside the sample. BGYF under UV, which is used as an indicator of AF presence in the industrial rapid screening techniques might not occur in all AF contaminated samples. Although UV control is not mandatory in Turkey, a field study carried out by Şahin (2013) [97] showed that 88% of dried fig processing facilities in Aydın used UV control. According to the results of another survey carried out at 91 dried fig processing facilities in Aydın in 2010 by Çobanoğlu (2012) [98], the average response for the use of UV-control against the AF contamination during the processing of dried figs was 2.48 (standard deviation=1.51, using a five point Likert scale, 1 (strongly disagree)-5 (strongly agree)). The processing steps continue with washing, oven drying, final visual inspection, fumigation, forming and packaging, respectively.

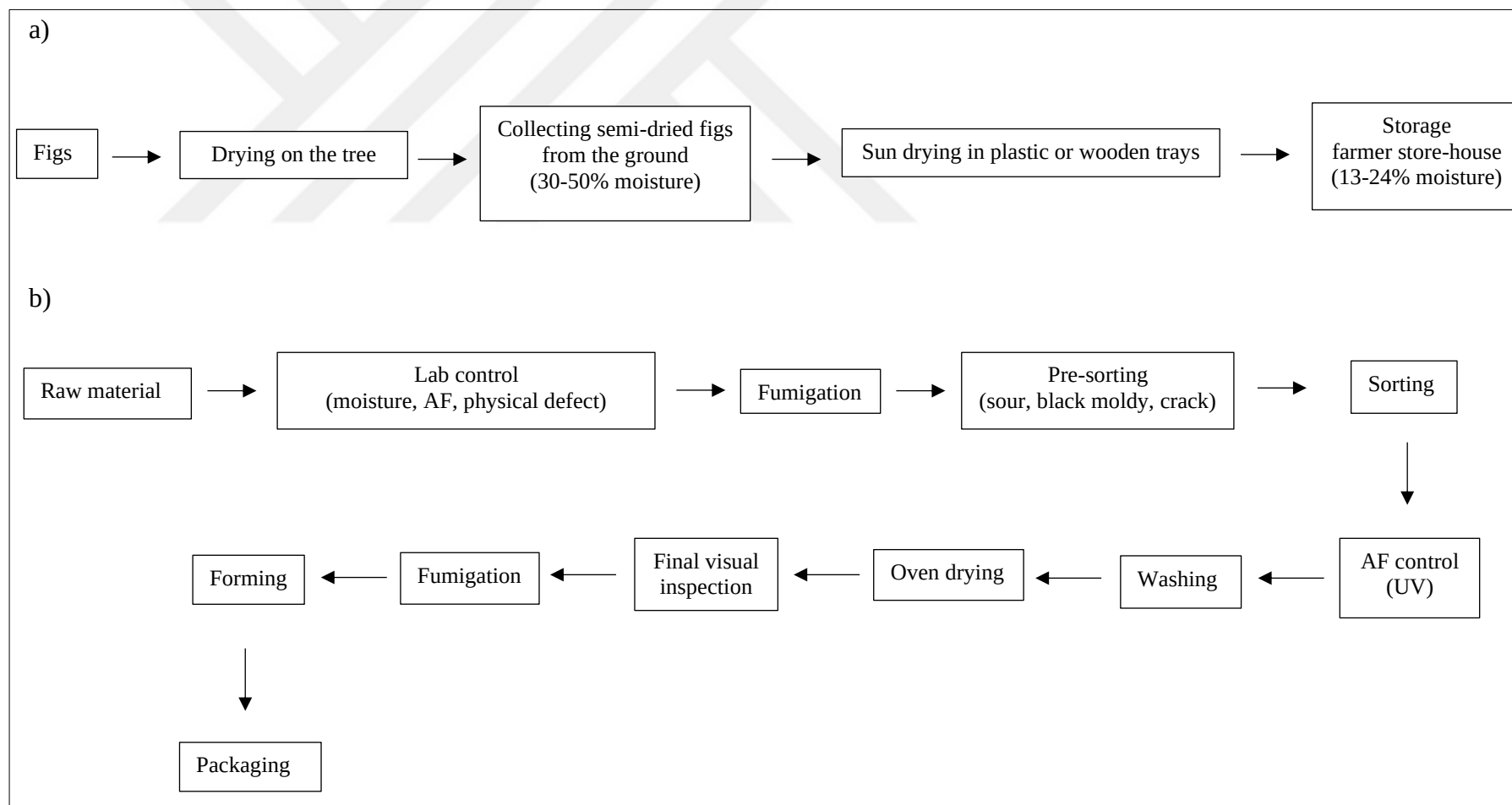


Figure 2. 2. Processing of dried figs at the orchard (a) and at the processing facility (b)

#### 2.2.4. Factors Affecting Aflatoxin Contamination in Dried Figs

AF contamination in dried figs can be affected by meteorological factors (weather conditions), agricultural and processing conditions. The factors affecting AF contamination of dried figs based on literature studies are shown in Table 2.8. Studies on the factors affecting AF contamination in figs are rather limited in literature. Code of Practice for Dried Figs published by Codex Alimentarius (CAC/RCP 65) [9] includes guidelines for the prevention and reduction of AF contamination considering each step of production (including site selection and orchard factors, caprification, pre-harvest, harvest, drying, transportation, storage, and processing) and the effect of weather related factors. The effects of meteorological and production/processing related factors are discussed in this section (Section 2.2.4.1 and 2.2.4.2).

Table 2. 8. Factors affecting AF content of dried figs

| <b>Meteorological Factors</b> | <b>Agricultural and Processing/Storage Conditions</b>        |
|-------------------------------|--------------------------------------------------------------|
| Temperature [8]               | Harvest method (hand harvest or collecting from ground) [58] |
| Rainfall [9]                  | Drying method [58]                                           |
| Humidity [9]                  | Pollination method [9]                                       |
| Wind [99]                     | Pre-sorting efficiency                                       |
|                               | Storage conditions<br>(Temperature, humidity) [9]            |

##### 2.2.4.1. Meteorological Factors

Meteorological factors such as temperature, rainfall, humidity and wind can affect AF contamination of dried figs during agricultural production and processing steps [8,100]. Higher contamination levels observed in drought years point to the effect of drought conditions observed during growing season (January-September) on AF contamination in dried figs [8]. AF contamination of dried figs were significantly higher ( $p < 0.05$ ) in 2007 (a

drought year) compared to 2003-2006, which was attributed to higher temperature (mean temperature of 20.3°C), lower rainfall and humidity conditions observed in (January-September) 2007 [78]. Drought conditions generally result in the formation of small wounds via insects and mites causing AF formation on the exterior and interior of dried figs [101]. Water stress caused by drought conditions can cause formation of free amino acids like proline and asparagine, which trigger the production of toxins by *A. flavus* and *A. parasiticus* [102].

Rainfall and humidity can increase the ostiole opening that can increase the risk of the development of fungi, resulting in an increase in AF contamination levels, and a decrease in the quality of figs [9]. Protection of the figs from rainfall and high humidity conditions during caprification and drying process is thus an important measure for the control of AF contamination.

Wind is another environmental factor that might affect AF contamination. Strong winds in late spring and early summer might cause wound formation due to friction in the shell of the fig fruit decreasing fruit quality [99].

#### 2.2.4.2. Agricultural and Processing Conditions

Agricultural, processing, and storage methods and conditions (during caprification, harvesting, drying and storage) might also affect AF contamination of dried figs. For example, profichi fruits, the figs used for caprification, should be free from fungi [9] and should be removed after caprification is completed (mid-July). Otherwise contamination can be spread by the caprification bees (*Blastophaga psenes* L). Harvesting and drying methods can also affect AF contamination. Harvesting with hand and solar drying system were found to be more effective than traditional techniques (collecting from ground/sun drying) [58]. Harvesting via collecting the samples from the ground causes the soil contact of dried figs while drying under sun may cause AF accumulation. Additionally in recent years, tunnel dryers has been adopted by the farmers in order to protect the sample from rainfall conditions [103]. The effect of tunnel drying on AF contamination in figs has not been studied yet.

In the processing facility, the most important part is pre-sorting step that removes the sour, black moldy, and cracked figs and then the UV control processing is applied in order to remove the AF contaminated dried figs using BGYF properties of AFs. Oven drying is an important step because inadequate drying can cause mycotoxin contamination during storage

(max 22-24%). Also, inappropriate packaging conditions can cause mycotoxin contamination when exposed to the humid conditions.

Storage method and conditions also affect AF contamination in dried figs. Temperature ( $<10^{\circ}\text{C}$ ) and moisture of storage rooms should be controlled in order to prevent contamination [9,100]. Also cull figs should be stored separately and should be free from insects. Storage using plastic crates instead of bulk storage decreases contamination risk by decreasing the probability of quality loss. Additionally, batch of dried figs should not exceed 1 m during storage [97] while in crates it should not exceed 30 cm in order to prevent fermentation and physical crushing [99]. Ozone treatment used during storage of dried figs for pest control also may result in degradation of AFs (such as AFB1 [104]). For example, degradation of AFB1 occurred with ozone treatment in 3 L exposure jar at 5 and 10 ppm for 3h and 5h [105].

## 2.3. PREDICTIVE MODELLING OF MYCOTOXIN RELATED HAZARDS

### 2.3.1. Data Sources

Main data sources for mycotoxin contamination of Turkish dried figs include scientific literature, databases of national and international control agencies and private control laboratories (Table 2.9).

Table 2. 9. Data sources for mycotoxin contamination of Turkish dried figs

| Data source                                        | Availability                                      | Data                               |
|----------------------------------------------------|---------------------------------------------------|------------------------------------|
| RASFF Portal                                       | Publicly available                                | Concentration ( $\mu\text{g/kg}$ ) |
| Scientific Literature                              | Public or private access (upon paid subscription) | Concentration ( $\mu\text{g/kg}$ ) |
| Food Safety Information System (of the Republic of | Not publicly available                            | Concentration ( $\mu\text{g/kg}$ ) |

|                                               |                        |                                       |
|-----------------------------------------------|------------------------|---------------------------------------|
| Türkiye Ministry of Agriculture and Forestry) |                        |                                       |
| Private Food Control Laboratories             | Not publicly available | Concentration ( $\mu\text{g/kg}$ )    |
| Aegean Exporters' Association                 | Publicly available     | Percent of AF contaminated dried figs |

RASFF is an information system established by food safety authorities in the EU to protect consumer health by providing rapid action on unsafe products imported to its members (member states of the European Union and Iceland, Switzerland, Liechtenstein, Norway) to quickly solve all kinds of food safety problems and inform the consumers via the RASFF portal [73]. The RASFF database includes detailed information about notifications reported by member countries such as classification of notifications based on the seriousness and the product distribution status on the market (alert, border rejection or information), date of case (month and year), sampling date, hazard category, notifying country, subject, product category, risk decision, action taken, and analytical results (Figure 2.3). Sample notifications are provided in Table 2.10. Notifications are evaluated considering the EU legal limits for the tested mycotoxins (AFB1 as  $6 \mu\text{g/kg}$  and total AF as  $10 \mu\text{g/kg}$  [3]). Although OTA is not regulated for dried figs in EU, the regulatory limit for dried vine fruits (raisins) of  $10 \mu\text{g/kg}$  is used for the control of OTA hazard in dried figs due to its potential carcinogenic properties.

The Republic of Türkiye Ministry of Agriculture and Forestry carries out routine mycotoxin analysis by sampling dried figs according to the “Turkish Food Codex of Mycotoxin Levels in Foods Declaration on Sampling, Sample Preparation, and Analysis Method Criteria for Official Control” (2018/10) during the periods it has determined and keeps the results in its own system, which is only accessible by Ministry employees working in that area. The sampling method for retail should be approximately 300 g for the official control of total AF and AFB1 levels in dried figs. If the retailer package contains more than 300 g dried figs (500 g-1 kg), the amount of aggregate sample changes based on the lot size. If the amount of dried figs in retailer package is smaller than 300 g one package can be a primary sample while the retailer packaging amount is much smaller, primary samples should contain two

packages. Also the sampling procedures change based on the lot size (Table 2.11). If it is not possible to apply this method due to the commercial problems which may be caused by the damage of the party which can be related to the transport and packaging forms, alternative methods can be used if the sample is as fully representative as possible and documented. The methods that will be used to detect the AF content of dried figs must be validated by interior and exterior laboratories which have met the performance criteria for specific concentrations (eg. 50-120% recovery for  $<1 \mu\text{g/kg}$  concentration of both AFB1 and total AF content).

Private accredited food control laboratories that meet the requirements determined by the Republic of Türkiye Ministry of Agriculture and Forestry (2018/10) also analyze mycotoxin content of dried figs in response to demand from the exporter companies and individual personal applications. Exporter companies are obliged to have mycotoxin analysis done for each lot in the Ministry's mycotoxin laboratories or private food analysis laboratories before exporting their products to the European Countries (E-21817801-305.04.02.02-3467821). The exporter companies can apply to the private food control laboratories that is published by the Ministry in the document “Export Procedure of Dried Fruit To The European Union Countries” (E-21817801-305.04.02.02-3467821) for the analysis the AF content of dried figs. Mycotoxin contamination data obtained by these laboratories are stored in their own databases that are only available to their employees.

In addition, the Aegean Exporters' Association determines the % of mycotoxin contaminated dried figs yearly considering their observations during the seasonal orchard controls and UV scan results of exporter companies after the first exportation stage is completed [106]. Research studies in the scientific literature (discussed in detail in Section 2.2.2.) also provide data on mycotoxin (mainly AF –total AF, AFB1, AFB2, AFG1), OTA, patulin, ergosterol, CPA, fumonisins, DAS, BEA, ENNs, FB1) contamination of Turkish dried figs based on samples obtained during different stages of the supply chain (i.e. agricultural production, processing, export, and retail).

Apart from Turkey's national mycotoxin monitoring, the European Union has a regulation (2017/625) for monitoring the mycotoxins in food and feed that is performed via collaborations of National Reference Laboratories of the EU Member States.

Search Page

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Notification

Reference

Subject

Notified by

Open alerts

Type

Type

Classification

Basis

Hazard

Category

Risk decision

Date

Week

Notified between

current week [29]

previous week [28]

week

of

year

01/01/2002

and

31/12/2019

(dd/mm/yyyy)

Product

Category

Flagged as

Country

Action taken

Keywords

Keywords

Open URL

Figure 2. 3. RASFF portal database [73]

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Table 2. 10. Sample notification from RASFF database

| Classification            | Date of Case | Notifyin Country | Subject                                                           | Product Category      | Type | Distribution Status                          | Risk Decision | Action Taken | Substance /Hazard | Sampling Date |
|---------------------------|--------------|------------------|-------------------------------------------------------------------|-----------------------|------|----------------------------------------------|---------------|--------------|-------------------|---------------|
| Border rejection          | 14/12/2018   | France           | AFs (B1 = 8.7; Tot. = 14.8 µg/kg - ppb) in figs paste from Turkey | Fruits and Vegetables | Food | Product not yet placed on the market         | Serious       | Destruction  | AFs               | 05/10/2018    |
| Information for attention | 29/11/2018   | Sweden           | OTA (134 µg/kg - ppb) in dried figs from Turkey                   | Fruits and Vegetables | Food | Distribution restricted to notifying country | Serious       | -            | OTA               | 23/11/2018    |

Table 2. 11. Sampling procedures used by The Republic of Türkiye Ministry of Agriculture and Forestry

| Commodity  | Lot weight (tonnes) | Weight or number of sublots | Number of incremental samples | Aggregate sample weight (kg) |
|------------|---------------------|-----------------------------|-------------------------------|------------------------------|
| Dried figs | ≥ 15                | 15-30 tonnes                | 100                           | 30                           |
|            | < 15                | —                           | 10-100 (*)                    | ≤ 30                         |

\*Changes based on lot weight

### 2.3.2. Modelling Studies

In the literature, empirical [14,107,108], mechanistic [10,12,109] and hybrid models [11,13] have been used for the prediction of mycotoxin especially AF-related risks in agricultural and food products (Table 2.12).

Empirical modelling generally explains the spatial distribution of the hazard (eg. AF contamination) using independent variables (eg. temperature, precipitation, humidity, characteristics of storage conditions) with the help of statistical models [110]. They are calibrated to predict the AF content using satellite-derived biophysical indicators, such as normalized difference vegetation index (NDVI) that gives information about the crop conditions was used in this study to predict the yield before harvesting [111,112]. With these models, predictions can be made based on new independent variables, but since the existing information about the processes is very limited, the correlation between independent variables varies, especially when time and area change, a very careful estimation should be developed [110]. For example, Boken et al., 2008 [108] carried out a study to predict the AF risk in peanuts using weather and satellite-based variables in West Africa (Mali). Considering the effect of drought conditions on AF they collected weather, yield and AF data for the region of Mali. NDVI used in this study to predict the yield before harvesting. The daily max, min temperature, precipitation, and sunshine hours of the 5 regions of Mali was obtained from the Direction National de la Meteorologie of Mali (1999) to determine the effect of weather variables on aflatoxin contamination. The AF content of the samples (determined by the ELISA test as 4.5-12.1 ppb) was collected in 1999. The yield data was used as a function of AF contamination considering the relation between drought, yield, and AF contamination. Then a crop simulation model was used in order to understand the duration of the peanut reproductivity phases. In this study, regression analysis was used to investigate the correlation between the amount of aflatoxin and weather variables as rainfall, average Tmax, Tmin, and sunshine times. Moderate relationship was observed between drought conditions and AF contamination in peanuts ( $R^2=0.56$ ).

In a study, authors tried to improve the predictive ability of an empirical model for the prediction of AF levels of corn samples in 2012 by focusing on the limitations due to uncertainties on the effect of environmental factors and production of aflatoxin and

locational differences in corn fields (South Carolina) [107]. In this study, daily temperature (obtained from NOAA's National Climatic Data Center), water activity, and field data was used as input variables using Gaussian process. They modelled AF production under controlled laboratory conditions considering different temperature and aw conditions. After that, in order to create a risk map (regional), the AF model was linked to the environmental conditions. While the accuracy of the model was not reported the uncertainty was quantified [107].

Another empirical model developed a drought index to predict the AF contamination in corn using two AF contamination datasets from the fields in Mississippi State (2000-2011, 2013 and 2014) and in South Georgia (1997-2004) by considering the effect of soil types and hybrids using multiple logistic regression [14]. In Mississippi, after hybrids (considering the susceptibility to AF) and soil types were selected the AF content of the samples ( $\mu\text{g/kg}$ ) was analyzed by a quick AF test kit. While 80% of the data was used to develop the model, 20% of data used for validation. In Georgia, 818 corn samples (total) were collected (1977-2004). While thin layer chromatography was used to detect AF contamination until 2000, quick AF detection test kit was used between 2000-2004. Similarly, 80% of data used to develop a model, 20% of data used for validation. The Agricultural Reference Index for Drought (ARID), which has been used in the literature to predict crop yields considering the effect of drought timing and drought degree, was used in this study to predict AF risk in corn. ARID values were calculated using meteorological data (daily max and min temperature, and rainfall data), which was obtained from the Agricultural and Forestry Experimental Station for Mississippi, while DAYMET database (1980 – 2004) and CRONOS database (1977 to 1979) were used for Georgia. Logistic regression was used for the prediction of AF contamination data, which was coded as contaminated (1) and not contaminated (0) using the maximum limit (USA) of  $20 \mu\text{g/kg}$ . Weekly ARID values were calculated before and after mid-silk (associated with high risk contamination) and then used as an independent variable in the logistic model to evaluate the change in AF contamination risk during the season. Type of soil, ARID values, and hybrid susceptibility were used to predict AF contamination using multiple logistic regression. The Mississippi model predicted 82% of the cases correctly while 97% accuracy was obtained using the Georgia model. Apart from these AF related studies, the occurrence of DON contamination in winter wheat was predicted using multiple regression [113]. Daily rainfall, daily minimum, and maximum air

temperatures, and hourly relative humidity (obtained from Canada weather station) was used as input data and DON concentration was predicted the variability in concentrations (73%) [113].

Mechanistic modelling includes a detailed understanding of the underlying causes of the hazard (eg mycotoxin contamination) such as biochemical behaviors of fungi and crops (eg. sporulation, germination, infection, fungal growth, toxin production rates etc.) [111]. In the mechanistic model, contamination conditions are estimated from laboratory and field data. Therefore, much more and detailed data is needed. In literature, in order to predict the daily AFB1 contamination in maize (from silk to harvest in the field) a mechanistic model was improved using four steps [10]. First *A.flavus* infection and AF production model prototype was developed by considering the infection cycle of *A.flavus*. For this, to define state and rate variables in the infection cycle and effect of weather data mathematical functions were developed using non-linear regression to calculate the AF cumulative index. Then the AF data with weather data was collected from 352 maize fields in Northern Italy (2005–2011) by considering the coordinates of the field as two different data sets (153 data in 2005-2008 and 199 samples in 2009-2011) and AFB1 content was determined ( $\mu\text{g/kg}$ ). Hourly temperature, relative humidity and rain data were obtained from agro-meteorological network in Emilia-Romagna. Then the exceeding probability of AFB1 legal limit of EU ( $5 \mu\text{g/kg}$  as  $0 (\leq 5 \mu\text{g/kg})$  and  $1 (\geq 5 \mu\text{g/kg})$ ) was determined using a logistic regression which developed using AF cumulative index in the prototype model. Then validation is performed and evaluated considering the internal (153 data in 2005-2008) and external data (199 samples in 2009-2011). While using regression 73% of contaminated maize (with AFB1 content higher than  $5 \mu\text{g/kg}$ ) obtained from the field was predicted correctly, the validation of external data demonstrated that the model predicted AFB1 contamination in maize with 68% accuracy[10].

In another study, authors were developed a mechanistic model to predict AF contamination in pistachio from meteorological parameters [12]. This study included 5 steps. First, growth stages of pistachio were determined using the Crossa-Raynaud Model then the prototype model is developed using the same framework of AFLA-maize model that is previously developed by Battilani et al., in 2003 based on the infection cycle of *A. flavus* [10]. While the daily temperature and rain data were obtained from [www.meteo.gr](http://www.meteo.gr), the daily relative humidity data were obtained from Greek National Meteorological Service for Aegina island

for the years 2014-2019. The data that obtained between 2014 and 2016 used for the growth stages while the data obtained between 2017 and 2019 used to validate the model. Pistachio samples were obtained from the Aegina Agricultural Association of Pistachio Growers (Sept-October, 2014-2019) and analyzed for AFB1 content in authorized laboratories. The probability function was then estimated based on the samples with AFB1 content higher than EU regulatory limit (12 µg/kg, EU 1881/2006 which was no longer in force) with a logistic regression as not contaminated if the concentration is lower than 12 µg/kg as 0 and higher than 12 µg/kg as 1 using 43 samples (2014-2016) considering the probability of the situation ( $p > 0.5$  (contaminated) or  $p \leq 0.5$  (not contaminated),  $p$ = probability). Then the model validated using 87 samples (2017-2019). Similar to the AFLA-maize study, the meteorological variables and infection cycle of *A. flavus* in the predictive model linked a mathematical framework to obtain AFB1 cumulative index which was the output of the model. To validate the model, the AF data obtained from the field study were compared with the model predictions and the model correctly predicted 95.6% of samples.

Hybrid models combine both mechanistic and empirical approaches. A hybrid AF risk prediction model improved [11] by considering the model which were improved by Chauhan et al., 2008 [13] by integrating the Agricultural Production Systems Simulator (APSIM) in maize samples before pre-harvest. APSIM enables the simulation of growth of maize with yield and soil water balance using daily meteorological parameters (max and min temperature, radiation, and rainfall). The model used 3 different temperatures for the growth of *A. flavus* and AF production in laboratory conditions. Three cardinal temperatures radiation, and rainfall data were obtained from Decagon (USA) automatic weather stations. During the grain filling, a drought index regarding the water demand ratio was used to simulate the growth of *A. flavus* and AF production. When the index was under the threshold ( $\leq 0.2$ ), model converted the index to the AF risk index (ARI) in order to predict the AF contamination as a function of temperature. To generate AF contamination data, experiments were performed at Kenya Agriculture and Livestock Research Organization using different maize hybrids planted in 2011 and 2012. Using a 2L of box, temperature and humidity were recorded following incubation and deep freezing, AF content of the grains were then determined using quick test kits (0-300 ppb). The prediction accuracy of the model was improved from 69% [13] to 0.74 - 0.94 using the hybrid approach with ARI [11].

Machine learning is a field of artificial intelligence that involves the development of algorithms that enable computers to learn from data and make predictions or decisions based on data [114]. Among the machine learning models commonly utilized for predicting mycotoxins in literature, the Bayesian network model stands out [115,116]. This probabilistic model combines expert knowledge with statistical methods, making it a powerful tool for predictions. While research focusing specifically on the use of machine learning to predict mycotoxin contamination risk has been limited [17,115,116], the field shows great promise for predictive modelling of food safety hazards. This potential is particularly apparent considering the complexity of the supply chain and scarcity of data within the sector.

For instance, a Bayesian network was used to demonstrate the relationships and interactions between food safety hazards for fruits and vegetables, and climatic, economic, and agronomic factors [117]. All fruit and vegetable-related food safety notifications originating from India, Turkey (countries with the highest number of notifications), and the Netherlands between 2005–2015 were obtained from the RASFF database. Relevant data on climatic conditions (such as temperature and precipitation), agricultural practices (including pesticide and fertilizer use), and economic factors (like price and production volumes) were collected for the studied time period and countries. Factors such as product category, notifying country, yearly production volumes, maximal residue level ratio, country of origin, and the annual agricultural budget of a country emerged as significant contributors to food safety risks (i.e. the total number of RASFF notifications). Surprisingly, the effect of meteorological factors was relatively low, which was attributed to the specificity of the relationship between food safety and climatic factors.

In another study [118], food safety hazards associated with cow's feed, including mycotoxin contamination in the Netherlands between 2000 and 2013 (sourced from the Dutch Quality Monitoring Program for Agricultural Products database), were predicted using a Bayesian network. A total of 17 variables related to climate and agrichemical were incorporated, achieving an overall accuracy of 90.3%.

In a study, authors developed a machine learning model utilizing the random forest algorithm. Crop phenology, weather and satellite data were incorporated to predict risks of various mycotoxins (DON, ZEN, T-2 and HT-2 toxins, fumonisins, AFB1, and OTA) in wheat samples in Europe on a regional basis [116]. The database encompassed 3115 wheat

samples, with DON presence data (112 data points) sourced from the Netherlands field survey [119,120], country-level data from the Grain Trader Association (185 data points), and Dutch Animal Feed Official Control Program (2818 data points) for the years 2010-2020. In the Netherlands field survey data, information about phenology was recorded and 1 kg of samples were collected by the farmers at harvest. Analytical assays were employed to determine DON content, while wheat field data were obtained from PDOK (<https://www.pdok.nl/>) spanning 2013 to 2020. Grain Trader Association post-harvest data was obtained through analytical methods and ELISA testing. Additionally, the Dutch Animal Feed Official Control Program conducted annual assessments of mycotoxin content using analytical methods. The average concentrations of the six mycotoxins and corresponding contamination levels were categorized as follows: low ( $\leq$ limit of quantification (LOQ) set at zero), medium ( $>$ LOQ and  $<$ threshold (i.e. one-tenth of the respective maximum limit or guidance value for the mycotoxin), and high ( $\geq$ threshold). The LOQs considering the analytical methods they used were DON (0.2 and 0.5 mg/kg), ZEN (0.05 mg/kg), OTA (0.002 and 0.025 mg/kg), AFB1 (and 0.005 mg/kg), FBs (0.1mg/kg), T2+HT2 (0.04 mg/kg). Crop phenology data were sourced from the World Food Studies growth simulation model while weather data (including Tmax, Tmin, total precipitation, mean vapor pressure and mean relative humidity) were obtained from Agri4Cast Data Portal ([agri4cast.jrc.ec.europa.eu/DataPortal/](http://agri4cast.jrc.ec.europa.eu/DataPortal/)). The developed model exhibited high prediction accuracy (0.90–0.99).

In 2021, Klimentia et al. introduced a risk assessment model that integrated three distinct machine learning techniques: Principal Component Analysis (PCA) for dimensionality reduction on the original dataset, Mahalanobis Metric for Clustering (MMC) for metric learning, and the k-nearest neighbors algorithm (KNN) for prediction. Their focus was on predicting AF contamination in dried figs based on the location and altitude of the fig orchards located in the Southern Peloponnese [17]. To quantify the presence of *Aspergillus* spp, soil samples were collected from 45 orchards during July-August 2013. Each orchard yielded six soil samples, which were subsequently subjected to inoculation and incubation. Colony-forming units (CFU) per gram of soil were then enumerated. The CFU results guided the classification of the orchards into five categories: very high ( $>$ 250 CFU/g), high, moderate, low or minimum (0-10 CFU/g). To determine the aflatoxigenic capacity of the fungus, a separate inoculation procedure was performed. The AF content was then quantified using a rapid test kit, yielding measurements between 0.0 and 113.1 ng/ $\mu$ l. Strains were

categorized into five groups based on their aflatoxigenic capacity: very high ( $>100$  ng/ $\mu$ l), high (20-100 ng/ $\mu$ l), medium (10-20 ng/ $\mu$ l), low (1-10 ng/ $\mu$ l), and non-contaminated ( $<1$  ng/ $\mu$ l). Classification of the orchards based on their AF risk yielded five groups ranging from very high ( $>400$  CFU/g ng/ $\mu$ l) to minimum (5 CFU/g ng/ $\mu$ l). By integrating the three methods (PCA-MMC-KNN), the developed model exhibited superior performance compared to the traditional KNN and PCA-KNN models [17].

Liu et al. (2018) conducted a study in which they compared the predictive performance of different models - empirical, mechanistic, and machine learning (specifically, Bayesian network) - for predicting DON contamination in winter wheat samples in the Netherlands [115]. In this study, same input and output data (encompassing weather data, agronomic data, and individual DON content of 625 wheat samples spanning the years 2001 to 2013) were used for all three models. The empirical model demonstrated the highest accuracy, achieving 88%, followed by the Bayesian network (86%) and mechanistic model (80%). The Bayesian network model exhibited the advantage of ease of implementation with limited input data.

In addition, the impact of climate change on mycotoxin contamination in agricultural and food products is an emerging food safety concern. While climate change can affect specific regions positively or negatively, global increase in average temperature and extreme weather events (eg. drought, precipitation) are expected in the future [121]. Predictive modeling has been used to investigate the impact of climate change on mycotoxin contamination. Mechanistic models (AFLA-maize and AFLA-wheat) were used to predict the contamination of AF in maize and wheat crops in the next 100 years under different climate change scenarios (+2 and +5 °C temperature increase) [109]. The input meteorological parameters (temperature, relative humidity, and the number of rainy days) were obtained from the MARS project (<http://mars.jrc.ec.europa.eu/>). Logistic regression was used to associate AF hazard index (AFI), the model output, based on daily climate data to 6 year field data on AF contamination of maize in Italy. A threshold AFI value greater than 95 was thus associated to the 50% probability of contamination above 5 $\mu$ g/kg [109]. Due to the limited field data for wheat no probability could be attached to the obtained AFI value. Therefore considering the legal limits of AFB1 in maize and wheat (5 and 3  $\mu$ g/kg, respectively) AFI $> 38$  was arbitrarily associated to the 50% probability of contamination above threshold. The model predicted AFB1 contamination in maize caused by a 2°C

increase in temperature, the most probable climate change scenario, to be a significant safety concern in Europe [109]. Another study determined the effect of climate change on DON contamination in wheat [122]. The meteorological parameters between 1975 and 1994 were used considering the changes in temperature, precipitation, etc. used for the future period (2031-2050) in north-western Europe and according to the results up to 3 times increase in DON concentration was expected [122].



Table 2. 12. Predictive modeling of mycotoxin risk in agricultural and food products

| Sample                  | Model                                                                                | Aim/Scenario                                                                    | Parameters                                                                                                                                                                          | Accuracy       | Ref  |
|-------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|------|
| <i>Mycotoxin Hazard</i> |                                                                                      |                                                                                 |                                                                                                                                                                                     |                |      |
| Maize                   | Hybrid model//Agricultural Production Systems Simulator (APSIM) model and risk model | Management scenarios to deal with dry and hot climates                          | Input data: Daily temperature, radiation, rainfall, soil water and soil nitrogen<br><br>Output data: AF risk index                                                                  | 69%            | [13] |
| Maize                   | Hybrid//APSIM and risk model                                                         | To develop an improved simulation model to predict pre-harvest AF risk          | Input Data: Daily temperature function for <i>A. flavus</i> growth and AF production using three cardinal temperatures (lab), radiation, rainfall<br><br>Output data: AF risk index | 99%            | [11] |
| Maize                   | Mechanistic model                                                                    | To predict the AFB1 contamination based on the <i>A. flavus</i> infection cycle | Input data: Hourly data of temperature, relative humidity and rain                                                                                                                  | 68% (external) | [10] |

|           |                   |                                                                                                                                                                           |                                                                                                                                                                         |       |       |
|-----------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-------|
|           |                   |                                                                                                                                                                           | Output data: AF cumulative index                                                                                                                                        |       |       |
| Pistachio | Mechanistic model | To develop a mechanistic, weather-driven model to predict <i>A.flavus</i> growth and the AFB1 contamination of pistachios on a daily basis from nut setting until harvest | Input data: Daily mean, max, and min temperature, relative humidity, and precipitation<br><br>Output data: AF cumulative index                                          | 95.6% | [12]  |
| Maize     | Empirical model   | To develop a methodology for generating probabilistic risk maps for unobserved quantities of AF                                                                           | Input data: Daily temperature, water activity, field data<br><br>Output data: AF cumulative index                                                                       |       | [107] |
| Corn      | Empirical model   | To predict the risk for AF contamination in corn using drought index                                                                                                      | Input data: Daily max, min temperature and dew point, precipitation, wind speed, potential evapotranspiration, and solar radiation<br><br>Output data: Probability (AF) | 56%   | [14]  |

|                            |                  |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                  |      |       |
|----------------------------|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-------|
| Peanut                     | Empirical model  | To monitor peanut contamination using normalized difference vegetation index                                                                           | Input data: Daily temperature (max and min), precipitation, and sunshine hours<br><br>Output data: AF concentration                                                                                                                                                                              | 0.56 | [108] |
| Dried Fig                  | Machine learning | To develop a risk assessment tool for the prediction of AF contamination                                                                               | Input data: <i>Aspergillus</i> spp. in the soil, soil pH, saturation percentage, electrical conductivity, organic matter, particle size analysis (sand, silt, clay), concentrations of Ca, Mg, K, Na, extractable P and trace elements (B, Fe, Mn, Zn and Cu)<br><br>Output data: AF risk factor | 85%  | [17]  |
| <i>Food Safety Hazards</i> |                  |                                                                                                                                                        |                                                                                                                                                                                                                                                                                                  |      |       |
| Fruit and Vegetables       | Machine learning | To demonstrate the relationship and interaction between the food safety hazards for fruits and vegetables, climate and economic, and agronomic factors | Input data: Climatic factors (monthly temperature, and precipitation), agricultural factors (pesticide use, fertilizer use), and economic factors (price, production volumes)                                                                                                                    | 95%  | [117] |

|            |                  |                                                                                                   |                                                                                                                                                                                                                                                                                    |               |       |
|------------|------------------|---------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|-------|
|            |                  |                                                                                                   | Output data: Hazard category                                                                                                                                                                                                                                                       |               |       |
| Cow's Feed | Machine learning | To predict the food safety hazard risk                                                            | <p>Input data: Climate data (17 variables) and agrichemical usage (6 variables)</p> <p>Output data: Probability (%)</p>                                                                                                                                                            | Overall 90.3% | [118] |
| Wheat      | Machine learning | To predict the risk levels (low-medium-high) of mycotoxins in wheat on a regional basis in Europe | <p>Input data: Mycotoxin monitoring data, wheat phenology data, daily weather data (max, min, and mean temperature, sum of precipitation (mm), mean vapour pressure (hPa) (Vp), and mean relative humidity (%) (RH)), and satellite images.</p> <p>Output data: AF risk levels</p> | 0.90–0.99     | [116] |

### 2.3.3. Decision Support Systems

Decision support systems and tools enable the results obtained in modelling studies to be interpreted and used to support decision making processes. However the modelling studies are rarely developed into digital systems that the actors can use before pre-harvesting to minimize mycotoxin hazard risk [17,123–125].

A crop simulation model was developed and validated in order to predict AF contamination in peanut using APSIM's peanut soil water and temperature module [123]. This model was then used as a decision support tool (AFLOMAN) by 8 peanut growers in the Kumbia, Wooroolin and Coalstoun Lakes regions of Australia. AF temperature factor was simulated based on the temperature content of soil (as  $<22^{\circ}\text{C}$ ,  $22\text{--}30^{\circ}\text{C}$ ,  $30\text{--}35^{\circ}\text{C}$ , and  $>35^{\circ}\text{C}$ ). When the crop was in the last pod-filling phase and the water content of soil was lower than 0.20, AF risk index (ARI) was calculated using the AF temperature factor. AFLOMAN interface was developed and the daily soil temperature was recorded by the farmers using pre-calibrated Tinytag1 data logger. A rain gauge was used to record the rainfall. Also, the program automatically took solar radiation and temperature data from the climate database (SILO 2009). The AF analysis was performed using the mini column qualitative method for rapid screening (detection limit 8 ppb) [126] after the peanuts were obtained from the growers. Then using regression, AF content was determined based on the ARI and observed AF content (mg/kg) relationship with  $R^2=0.92$ . The farmers obtained the AF risk report via email upon request.

Apart from this study, there are two important European Union projects – MycoKey project, which resulted in the development of an integrated system to minimize mycotoxin risk in cereals and maize globally and MyToolBox project [124], which developed a platform that combined information from each step of the supply chain (pre-harvest, post-harvest, and processing) for wheat, barley, fig, and peanut to minimize mycotoxin risk. The integrated system developed by the MycoKey project enabled monitoring, characterization, prevention, intervention and remediation of the mycotoxin risk throughout the supply chain [127]. The one of the specific objectives of this project was to integration of the available predictive models for AF, fumonisins, DON, and ZEA in maize sample to minimize the mycotoxin risk. Tox-Maize model was developed for mycotoxin management to quantify the effect of

different ecological conditions on mycotoxin production during the project. Validation was performed using data obtained by a field study and meteorological data (temperature, relative humidity, and rain) obtained from the stations in 2016 and 2017 which is close to the maize area (in Italy). The AFB1 contamination and the predicted data from AFLA-maize, FER-maize, and Tox-Maize were compared and Tox-maize model provided 20% fumonisin reduction in the probability of maize contamination, while the results for AF was not good.

MyToolBox project developed a platform to minimize mycotoxin risk via recommendations based on the expert knowledge. One of the specific outputs of this project was to develop a platform to manage mycotoxin risk by the actors in the sector [128]. In this project, the amount of maize considering the mycotoxin contamination levels (as  $<5 \mu\text{g/kg}$ ,  $5\text{-}20 \mu\text{g/kg}$ , and  $>20 \mu\text{g/kg}$ ) of AFB1 from weather data to be used by farmers before pre-harvest was predicted using the *Aspergillus* resistant cultivars [130]. According to this model, if 80% of farmers use the *Aspergillus* resistant maize cultivars, maize contamination (i.e. maize with AFB1 concentration  $>20 \mu\text{g/kg}$ ) would decrease by up to 9%.

### 3. EVALUATION OF RAPID ALERT SYSTEM FOR FOOD AND FEED (RASFF) DATA FOR MYCOTOXIN CONTAMINATED DRIED FIGS ORIGINATING FROM TURKEY

#### 3.1. INTRODUCTION

The fig (*Ficus Carica* L.) is a fruit of high nutritional value, typically stored and consumed in dried form to extend its shelf life. Turkey holds a significant position as the producer of 56% of the world's dried figs, making it the largest dried fig exporter (with 88,509 tonnes exported in 2020) [129]. Over the last two decades, France and Germany have been the top two importers of dried figs from Turkey [130].

The quality of dried figs can be affected at various stages: on the farm (during pre-harvest and harvest), in the processing facility (during processing and storage), and during transportation [6]. The drying of figs begins on the tree. Semi-dried figs, with approximately 30-50% moisture content, fall to the ground and are collected by farmers for sun drying using plastic or wooden crates [57]. Once drying is completed, according to the Turkish dried fig standard [96], the farmer classifies the product into categories: “extra” (highest quality dried figs), “grade I”, “grade II”, and “industrial” (lowest quality product). This classification is based on factors such as moisture content, color, and the ratio of defective dried figs (e.g. burnt, bird-stained, etc.) in a one kg product (R. Konak personal communication). Farmers sell the dried figs to the tradesmen, who typically sell the product to warehouses or directly to exporting companies. The exporter companies purchase dried figs from the warehouses throughout the year based on export demands, or directly from the farmers at the start of the harvest.

One of the main safety hazards in dried figs is AFs, which are produced in warm and humid conditions, primarily by *Aspergillus flavus* and *Aspergillus parasiticus* [131]. AF contamination can occur on the farm (during pre-harvest and harvest), in the processing facility (during processing and storage), and during transportation [6,57]. AFs (B1, B2, G1, G2), the main mycotoxins in dried figs, are highly toxic dietary risk factors [1] and are classified as “carcinogenic compounds for humans” (group 1) according to the International Agency for Research on Cancer [132]. Currently, the EU has established maximum

regulatory levels for AFs in dried figs: 6 µg/kg for AF B1 (AFB1) and 10 µg/kg for total AF (including B1, B2, G1, G2) [3].

Over the years in Turkey, modifications in the production, processing, storage and transport conditions of figs aimed at minimizing the safety risks associated with AFs (such as alterations in the caprification process, usage of crates, drying tunnels, and covers under the trees) have been implemented. These changes were based on work by the Aegean Exporters' Association [106] and the Republic of Türkiye Ministry of Agriculture and Forestry (specifically by the Fig Research Institute in Aydın) [133].

In Turkey, dried figs are exported after the separation of AF-contaminated dried figs in the processing facilities. This is achieved through manual selection under ultra-violet (UV) light, taking advantage of the fluorescence attributes of AFs under 360 nm [28]. However, manual selection under UV light may not adequately control AF risk in dried figs, as evidenced by the high number of notifications observed in the RASFF portal of the EU [73].

In 2018, the EU carried out a food safety audit to examine the control system in Turkey for dried figs intended for export [134]. While the audit acknowledged the presence of the necessary legal and organizational framework, it noted a lack of promotion of good manufacturing practices in processing and distribution sectors. It also highlighted shortcomings in control measures such as sampling and health certificates, and the inadequacy of local investigations into non-compliant consignments in the EU import controls [134].

The RASFF portal was established in 1979 by the EU under the Regulation (EC) 178/2002 to protect consumer health and provide an emergency response for all kinds of food safety risks [135]. The RASFF portal serves as an effective tool to inform EU authorities about food safety risks, enabling quick responses and preventing the entry of risky food and feed into member countries. Each RASFF notification includes classifications based on the seriousness of the risk and the product distribution status on the market (alert, border rejection, or information), product category, action taken, date of the case (month and year), sampling date, hazard category, risk decision, notifying country, subject, and analytical results [73].

The RASFF database provides valuable information about food safety risks, which can be used to understand their frequency and associated trends [136]. In literature, RASFF data

has been analyzed considering product categories such as seafood products [137], herbs and spices [138], fish products [139,140], meat samples [141], fruits and vegetables [117], and hazard categories such as biogenic amines [142], foreign bodies [143], heavy metals [144], food allergens [145], food additives [146], food fraud incidents [147], foodborne pathogens [148], mycotoxins [149–151], and AFs [152] to investigate food safety risks.

Analysis of mycotoxin hazard notifications for border rejections between January 2008 and October 2010 showed that 43% of all notifications were related to pistachios originating from Iran. This was followed by various products from Turkey, including hazelnuts, dried figs, pistachios, and raisins, which accounted for 17% of notifications [149]. Pop et al. (2015) analyzed RASFF annual reports between 2007 and 2014 for mycotoxin hazards in food and feed. They reported that 4600 out of 5006 notifications were related to high level of AF. This was followed by OTA (289 notifications), deoxynivalenol (DON) (52), and fumonisins (39). Almost every year, Turkey was a notable offender due to the mycotoxin levels found in pistachios, nuts and derived products, dried figs, and raisins [150].

Mycotoxin hazard data from the RASFF portal between 1981 and 2017, covering 10,912 notifications, showed that the majority were related to AFB1 contamination (80%), followed by total AF (13%), OTA (5%), fumonisins and DON (0.5-1%), and AFM1, citrinin, patulin, T-2, and ZEA (< 0.5%) [151]. Turkey was the second most notified country of origin for AFB1 (with 1,868 notifications) and total AF (227 notifications) after Iran (1,882 notifications for AFB1 and 682 notifications for total AF). However, Turkey was the most notified country for OTA hazards, with 120 notifications [151].

RASFF data specifically for Turkey was analyzed for all hazard categories between 2009-2016 [7] and by product categories between 2009-2018 [153]. The most notified hazard category for dried fruits and vegetables, nuts, and seeds was mycotoxins (48%) [7]. AFs (89%) and OTA (9%) were reported as the most predominant mycotoxins [153].

Despite the significant food safety risk posed by mycotoxins, especially in foods originating from Turkey like nuts, dried figs, and raisins, the mycotoxin hazard in Turkish products in general, and specifically in dried figs, has not been extensively investigated. This study aims to fill this gap by systematically analyzing RASFF notifications for dried figs originating from Turkey. A comprehensive approach was used to investigate factors that affect RASFF notifications (including the total number of notifications and the mean total AF

concentration). Factors that influence mycotoxin contamination such as weather conditions, agricultural and processing conditions, as well as other non-product related factors, such as export quantities and regulatory changes were taken into consideration for this analysis.

## **3.2. MATERIALS AND METHODS**

### **3.2.1. Creation of the Database**

Data on mycotoxin hazards in Turkish dried figs were sourced from the RASFF Portal [73]. Each notification included information on the date of the case, notification type (alert, border rejection, and information), notifying country, action taken, risk decision and the concentration of mycotoxins. Notifications related to dried figs exported from Turkey to the EU were filtered by selecting the product's origin as "Turkey" and the hazard category as "mycotoxins". The scope of notifications was further narrowed down using the keyword "fig".

Given that the export of dried figs from Turkey to the EU generally begins after mid-September (C. Işlak personal communication), a "production year" was defined, for analysis purposes, as the 12-month period starting in mid-September and ending in mid-September of the following year. A total of 1,052 notifications (including 967 dried figs, 33 dried fig pastes, 31 organic dried figs, 18 diced dried figs, and 3 boiled dried figs) for mycotoxin hazard (975 AFs and 77 OTA) reported from 15/09/2002 to 15/09/2020 were included in the analysis. All fig products including fig paste, diced dried figs, and boiled figs, were analyzed together. A database containing all available data for the 1,052 notifications was created using Microsoft Office Excel spreadsheets for each production year (Microsoft, USA).

### **3.2.2. Analysis of RASFF Data**

Descriptive statistics were used to summarize the data. Visualization of the number of notifications using bar and line charts was done in Microsoft Office Excel (Microsoft, USA) to determine monthly and yearly trends.

To investigate the relationship between the number of notifications and the amount of exported dried figs, monthly export data was obtained from the Turkish Statistical Institute (TUIK) for 19 RASFF members (France, Netherlands, Germany, Italy, Denmark, Greece, Portugal, Spain, Belgium, Luxemburg, Norway, Sweden, Finland, Austria, Malta, The Czech Republic, Slovakia, Romania, and Bulgaria) for the investigated production years [130]. Linear regression analysis was conducted using SPSS to evaluate the effect of export quantities on the total number of notifications.

To understand the impact of mycotoxin concentration on monthly and yearly trends, total AF, which includes AFB1, AFB2, AFG1, and AFG2, was selected for further analysis. For the concentration of total AF ( $\mu\text{g/kg}$ ), mean, minimum, and maximum concentrations were determined. For each notification, AF concentration data exceeding the maximum regulatory level was used to calculate the mean. The mean concentration of total AF was tabulated for each year (Table 3.1) and month (Table 3.2).

The correlation between the total number of RASFF notifications and the percentage of AF-contaminated dried figs reported by the Aegean Exporters' Association [106] (Figure 3.1) was tested to understand the effect of mycotoxin contamination levels on notification trends. The Aegean Exporters' Association determines the percentage of AF-contaminated dried figs produced in Turkey each year within the scope of the "Disposal of Dried Figs Contaminated with AF" project, utilizing data on the quantity of dried figs received, separated as AF-contaminated, and exported. Literature data on mycotoxin contamination of Turkish dried figs (Table 2.3) were also evaluated to explain the observed trends. Seasonal Dried Fig Yield Estimation Reports (from 2004 [154] until 2018 [155]) published annually by the Aegean Exporters' Association Committee, based on the evaluation of fig orchards in Aydın and İzmir, were reviewed to gather information on factors affecting product quality.

### **3.3. RESULTS AND DISCUSSION**

#### **3.3.1. Descriptive Statistics**

The analysis of RASFF notifications from 2002 to 2019 was conducted to determine the notification types, notifying countries, risk decisions, and actions taken. The most common RASSF notification type for Turkish dried figs was 'border rejection', with 729 notifications

(69%), followed by ‘information’ with 233 notifications (22%), ‘information for attention’ with 46 notifications (4%), ‘alert’ with 43 notifications (4%), and “information for follow up” with one notification. Border rejection notifications are issued when significant health risks are detected in food samples at border controls, leading to product rejection. Alert notifications are used when there is a serious risk situation in the market requiring rapid action. Information notifications are used when the products are not on the market yet and pose no health risk, thus no immediate action is needed [156].

Among the 27 notifying countries, France and Germany had the highest number of notifications (27% and 26%, respectively), followed by Italy (11%), and Denmark (6%). According to TUIK data [130], France and Germany were the top two exporters of Turkish dried figs between 2002 and 2019, suggesting a correlation between the export volume of dried figs and the number of notifications.

The mycotoxin hazard risk in Turkish dried figs was categorized as “undecided” until 2012. Between 2012 and 2020, except for four notifications, the risk was categorized as “serious”. This change in risk categorization is due to a new feature added to the RASFF portal database in 2012 to determine the risks associated with notifications [157]. “Re-dispatched” was the most common action taken for notified Turkish dried figs, with 475 notifications (45%), followed by "placed under custom seals" with 136 notifications (13%), "official detention" with 100 notifications (10%), "returned to the consignor" with 80 notifications (8%), "import not authorized" with 68 notifications (7%), "destruction" with 48 notifications (5%), and "withdrawal from the market" with 43 notifications (4%).

### **3.3.2. Notification Trends**

To use the total number of notifications as an indicator of product quality (i.e. mycotoxin contamination), the influence of non-product related factors (such as export data, and regulatory changes) need to be considered and, if possible, eliminated. A significant regulatory change occurred in November 2012, when the EU increased the maximum limits of AFB1 and total AF in dried figs from 2 µg/kg to 6 µg/kg, and 4 µg/kg to 10 µg/kg, respectively [3]. This change was accounted for when interpreting the trends.

The annual number of notifications for mycotoxin hazard in Turkish dried figs ranged from 11 to 117 for the production years of 2002-2019 (totaling 1052) (Figure 3.1). The monthly distribution showed the highest number of notifications in November (333 notifications, 32%) in 14 out of the 18 years, followed by January, December, and October (180 (17%), 177 (17%), and 128 (12%) notifications, respectively) (data not shown). The high number of notifications in these four months can be explained by the harvest season that begins in mid-September and the peak export periods of dried figs. According to TUIK's monthly export data [130], the three highest exporting months were September, October and November in the years 2004-2006, 2010-2013, 2016, 2018-2019, and October, November, and December in the years 2002-2003 and 2007-2009, 2014-2015, and 2017.

The relationship between yearly export and notification data was evaluated using linear regression. Since the effect was not significant ( $R^2=0.120$ ,  $p>0.05$ ), this relationship was not further considered. Factors affecting product quality/mycotoxin contamination (eg. weather conditions, agricultural practices, and processing applications) are explored further in the subsequent discussion.

The trends in % AF-contaminated dried figs in Turkey reported by the Aegean Exporters' Association [106] and total number of RASFF notifications appear similar, suggesting a relationship between the extent of product contamination and the RASSF notifications. However, the correlation was not significant ( $r=0.419$ ,  $p>0.05$ ).

The two maxima observed in 2007 and 2012 (Figure 3.1) warrant special attention. The maxima in 2007 coincides with the highest mycotoxin contamination levels of Turkish dried figs reported in the literature for the years 2003-2007 [8,78]. While the percentage of mycotoxin-contaminated dried figs ranged from 2.4 to 4.6% between 2003-2006 [8,78], the contamination level peaked at 9.8% in 2007 [8]. This increase was attributed to drought conditions that led to a rise in the mean temperature (1-2°C) with less precipitation (300 mm), and relative humidity (10-15%) [8].

Drought conditions observed in 2007 [158] appear to contribute to the high number of notifications reported for this production year (Figure 3.1), likely due to their effect on mycotoxin contamination. Weather conditions such as drought, wind type, and precipitation might increase AF contamination of dried figs, primarily due to damage to the fig skin structure, such as cracking of the fig fruit, small wounds on the surface of dried figs, and

ostiole-end splitting [8,159]. Long-term drought conditions also stimulate AF production by *A. flavus* and *A. parasiticus* due to the high levels of proline amino acid, which stimulates the secondary metabolism of microorganisms in drought-stressed plants [102,160].

The maximum number of notifications observed in 2012 (117) also coincides with drought conditions observed in the main fig producing regions of Turkey [161,162], resulting in cracks and sunburn diseases, providing further evidence of the impact of drought conditions on mycotoxin contamination. The EU regulatory change in 2012 may have partially contributed to the sharp decrease in the total number of notifications in 2013 (a 66% decrease, down to 40) accompanied by a 36% increase in the mean total AF concentration (up to 38 µg/kg).

Product quality problems caused by weather conditions were reported in Turkey in 2013, 2017 and 2018 [155,163,164]. The year 2013 was characterized by high temperatures and wind conditions at the beginning of August, causing sunburn disease in some orchards [163]. In 2017, cracking was observed in the first fig harvest due to rain and high humidity; sunburn disease was also observed in orchards where pruning was not done correctly [164]. Widespread problems were reported in product quality in 2018 at the first harvest due to unbalanced weather conditions such as rain, intense precipitation, and dew conditions [155]. However, these quality problems are not entirely reflected in the RASFF notification or % AF contamination data.

The second-lowest number of notifications observed in 2018 (24) (after 2003) coincides with the lowest % of AF-contaminated dried figs value reported by the Aegean Exporters' Association (0.4%) (Figure 3.1). Similarly, for the production year 2019, reports of higher product quality (i.e. lower rate of cracked and sunburned dried figs) than in 2018, and winter weather conditions within seasonal norms [93], fail to correlate with the total number of notifications. The interpretation of the observed (decreasing or increasing) trends in the number of notifications requires an in-depth statistical analysis of the weather data over the investigated time frame, with careful consideration of other relevant factors. Such analysis falls outside the scope of this study.

An in-depth review of sectoral reports reveals important insights into specific agricultural and processing practices that may have impacted mycotoxin contamination levels in dried figs. Reported problems in pollination and collection practices might have contributed to the

mycotoxin contamination levels observed between 2004 and 2007. Pollination should be performed from the beginning to the end of June; after pollination, profichi fruits should be removed from the orchards to prevent AF contamination via *Blastophaga psenes* L. [165]. Research by the Aegean Exporters' Association revealed that the profichi fruits in nets/cartons were not collected from the trees on time in 2004, 2005, 2006, and 2007 [154,158,166,167]. The frequency of semi-dried fig fruit collection from the ground is another factor that might impact mycotoxin contamination. According to The Yields of Fig Estimation Reports of the Aegean Exporters' Association, dried figs were collected from the ground once every 2 or 3 days by the farmers between 2004-2007 [154,158,166,167], instead of daily as recommended to decrease soil contact of dried figs. The delays in collection of nets/cartons and collection frequency could have contributed to mycotoxin contamination levels.

While crates started to be used in 2004 in Turkey, their widespread adoption by farmers took place in 2009 after their quality was improved by the Aegean Exporter's Association. The use of crates as an alternative to drying on grass or soil is expected to have a positive effect on mycotoxin contamination after 2009 [168]. The yield losses observed due to poor caprification practices (i.e. incorrect caprification timing) and significant *Lonchaea aristella* damage observed in some orchards might have contributed to mycotoxin contamination in 2011, a year characterized by regular precipitation patterns [169].

The widespread use of drying tunnels since 2018 by fig producers, first distributed to organic fig producers in 2017 by the Aegean Exporter's Association [103], and harvesting with a cover under the tree [170], might have improved the quality of dried figs, thereby reducing mycotoxin contamination. The increased use of drying tunnels, crates, harvesting with cover, and limited pollination problems due to differences in ripening periods in high altitude areas in 2019, were likely to have a positive impact on product quality [93]. While these practices might have impacted contamination levels to varying levels, these effects are not quantifiable.

The mean total AF concentrations ranged between 13  $\mu\text{g/kg}$  and 44  $\mu\text{g/kg}$  (Table 3.1/Figure 3.1). The wide variation between the annual number of notifications (2-106) and the presence of significant outliers in certain years (such as 2012) limit the usefulness of mean concentration data for trend analysis. Yet, several important observations can still be made. Firstly, the impact of drought conditions in 2007 and 2012 was not reflected in the mean

concentration data. Secondly, an increase was observed in mean concentrations after 2012 (in the range of 28-44  $\mu\text{g}/\text{kg}$  between 2012 and 2019, Figure 3.1), which could partly be attributed to the regulatory change in the limit of mycotoxin contamination in the EU that took effect in 2012 [3].

AF contamination can occur throughout dried fig production (Table 2.3), yet there is no data to suggest that higher AF concentrations in dried figs are associated with specific contamination routes. According to an article published by Özay & Alperden in 1991, contamination of mycotoxins in dried figs begins on the tree and can continue during storage due to poor drying and storage conditions. The comparison of contamination levels at different time points during dried fig production is further complicated by sampling difficulties [57] due to limited sample size for each processing step.

The study of AF development (from pre-harvest to product) in different food samples (peanuts, brazil nuts, tree nuts other than Brazil nuts and pistachios, pistachio nuts, maize) provides important insights into the time course of AF formation in food samples and the factors affecting it [171]. Insect damage, drought stress, and rain observed in pre-harvest, slow drying observed during the post-harvest, poor storage and processing conditions such as processing without sorting, and inadequate and improper control practices can all increase AF concentration [171]. Only good practice in processing steps decreases AF concentration.

Similarly, AF contamination might occur in each step of the dried fig production process. While insect damage, drought conditions, and rain can increase AF contamination during pre-harvest [8,102], drying conditions (rapid or slow drying) also affect the level of AF contamination during post-harvest (i.e. slow drying and drying on the ground increase AF concentration, weather conditions during drying can influence the contamination positively or negatively). Improper storage and processing conditions (such as processing without sorting, processing without UV control, and inefficient drying after washing) and poor laboratory analysis control (moisture, AF, physical defect) can increase AF contamination. The final concentration in the product is a consequence of the cumulative effect of contamination at each step and the inefficiency of the steps used to control the contamination. Therefore, the highest AF concentration can be observed in the storage step.

### 3.4. CONCLUSION

A general analysis of RASFF data related to mycotoxin hazard in Turkish dried figs was carried out considering the date of the case, type of notification (alert, border rejection, information), notifying country, action taken and risk decision. Factors that affect mycotoxin hazard (eg. weather conditions, and agricultural and processing conditions) and other non-product related factors (eg. export quantities, regulatory changes) were evaluated considering the yearly and monthly notification trends. Mycotoxin contamination might occur at each step of dried fig production such as pre-harvest, post-harvest, processing, and storage. Particularly, drought conditions observed in 2007 and 2012 contributed to the high number of notifications reported for these production years (104 and 117 notifications, respectively). However, a similar change was not observed in the mean total AF concentrations, which ranged between 13 µg/kg and 44 µg/kg with a wide variation between the annual numbers of notifications (2-106).

While the findings of this study suggest a correlation between weather conditions (such as drought) and mycotoxin risk in dried figs, future research on predictive modeling of this relationship is warranted. The ability to predict mycotoxin safety risk under different weather scenarios such as temperature, precipitation, and extreme weather events etc. would contribute greatly to the control of mycotoxin risk in dried figs, particularly in the context of climate change.

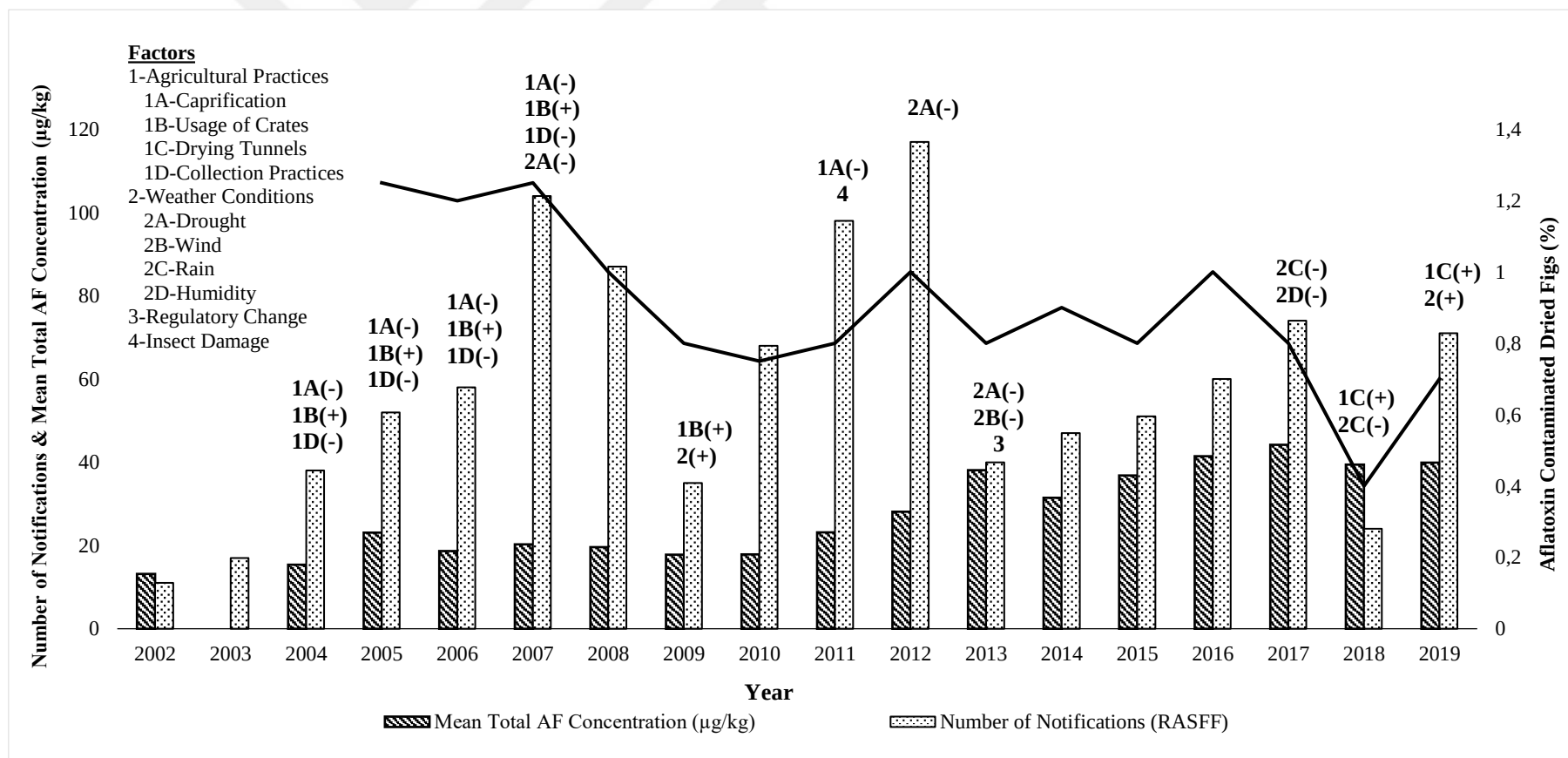


Figure 3. 1. Trends in RASFF data for mycotoxin hazard in Turkish dried figs (2002-2019) (total number of notifications and mean total AF concentration ( $\mu\text{g/kg}$ ) data) (including the Aegean Exporters' Association data on percentage of AF contaminated dried figs (2005-2019) [106]) (Factors that might affect the notification trends indicated as positive (+) or negative (-))

Table 3. 1. Total AF content of Turkish dried figs by year (calculated using RASFF data)

| Year | Mean    | SD    | Min     | Max     | N   |
|------|---------|-------|---------|---------|-----|
|      | (µg/kg) |       | (µg/kg) | (µg/kg) |     |
| 2002 | 13.16   | 6.49  | 4.2     | 25.3    | 8   |
| 2003 | 73.95   | 11.24 | 106.6   | 114     | 2   |
| 2004 | 15.40   | 16.97 | 3.3     | 96.8    | 37  |
| 2005 | 23.10   | 29.66 | 3.4     | 139.4   | 49  |
| 2006 | 18.64   | 20.27 | 3.5     | 128     | 52  |
| 2007 | 20.27   | 29.92 | 3.1     | 215.2   | 98  |
| 2008 | 19.63   | 20.78 | 3.9     | 129.1   | 81  |
| 2009 | 17.80   | 20.04 | 3.6     | 130     | 34  |
| 2010 | 17.85   | 17.91 | 2.7     | 113     | 61  |
| 2011 | 23.18   | 24.44 | 3.8     | 134.12  | 87  |
| 2012 | 28.11   | 29.44 | 4       | 400     | 106 |
| 2013 | 38.09   | 25.08 | 12.34   | 130.3   | 25  |
| 2014 | 31.48   | 18.47 | 12.7    | 86      | 24  |
| 2015 | 36.82   | 29.14 | 10      | 169     | 40  |
| 2016 | 41.43   | 39.71 | 5.38    | 206     | 47  |
| 2017 | 44.19   | 56.55 | 9.5     | 360     | 67  |
| 2018 | 39.47   | 30.21 | 10      | 114     | 17  |
| 2019 | 39.89   | 40.08 | 10      | 263.69  | 56  |

Table 3. 2. Total AF content of Turkish dried figs by month (calculated using RASFF data)

| Month     | Mean    | SD    | Min     | Max     | N   |
|-----------|---------|-------|---------|---------|-----|
|           | (µg/kg) |       | (µg/kg) | (µg/kg) |     |
| January   | 24.00   | 22.24 | 3.1     | 139.4   | 149 |
| February  | 35.53   | 56.47 | 4       | 360     | 45  |
| March     | 33.01   | 30.18 | 4.4     | 107.6   | 26  |
| April     | 18.30   | 17.32 | 3.3     | 81.1    | 34  |
| May       | 25.68   | 28.53 | 3.5     | 114     | 19  |
| June      | 28.01   | 26.92 | 4.7     | 114     | 18  |
| July      | 20.97   | 19.78 | 6       | 133.4   | 20  |
| August    | 26.15   | 28.22 | 4.1     | 106.6   | 16  |
| September | 15.42   | 16.79 | 3.6     | 64      | 13  |
| October   | 24.59   | 26.07 | 3.5     | 400     | 112 |

|          |       |       |     |       |     |
|----------|-------|-------|-----|-------|-----|
| November | 27.68 | 32.98 | 2.7 | 288.7 | 285 |
| December | 31.53 | 36.18 | 3.9 | 215.2 | 154 |



## 4. PREDICTING MYCOTOXIN CONTAMINATION OF TURKISH DRIED FIGS FROM METEOROLOGICAL FACTORS

### 4.1. INTRODUCTION

AFs, mycotoxins produced by the fungal species *A. flavus* and *A. parasiticus*, pose a significant food safety hazard for various food products, including dried figs. Environmental conditions, such as temperature, precipitation, and humidity, have a significant effect on the life cycle of mycotoxigenic fungi and mycotoxin production, and therefore mycotoxin contamination during the production and processing stages of food products [172,173]. However, the specific impacts differ based on the type of mycotoxin, the specific food product, and the stage of production, processing, and storage [171,173].

Fig is a subtropical plant that grows in regions where the annual average temperature ranges between 18-20°C. Temperature, specifically drought conditions [8], rainfall and relative humidity [9] are the principal meteorological factors influencing AF contamination in dried figs. The utilization of predictive modeling serves as an effective tool in managing and reducing the risk of mycotoxin hazards [10,11,174,175]. Traditional methods employ mechanistic, empirical, and hybrid models to predict mycotoxin risk across various agri-food samples [111]. While there was no any traditional modelling study that predict the mycotoxin contamination in dried figs using meteorological parameters, some of these modelling studies predicted mycotoxin risk using meteorological parameters [10,11,108] in different agri-food samples with some limitations. For example, an empirical model demonstrated that moderate relationship ( $R^2=0.56$ ) between drought conditions and AF contamination in peanut which determined using regression analysis. But, the yield data used as an indicator of AF contamination in this study and the yield data was not available based on the district level. Another study [10] was also used the weather conditions for the prediction of AF in maize by mechanistic model and imprecise AF production obtained due to the lack of field data however 73% of contaminated maize samples predicted correctly with regression. A hybrid modelling study [11] used the meteorological parameters for the prediction of AF risk in maize using regression analysis and the water demand ratio was added to the model that takes into account drought that means moisture availability in the soil. The 99% of the variation explained by this model in a linear relationship. Logistic

regression and multiple regression, traditionally most frequently utilized methods, which uses the linear combination of independent variables [176]. However, data scarcity, non-linear and time dependencies and prediction of out of sample situations present challenges for prediction using traditional models and could be addressed using machine learning approaches. Machine learning approach was developed to predict the mycotoxin hazard risk in agri-food considering the possibilities of methods allows the non-linear and time dependencies without using full dataset via learning from data and make predictions and decisions. In existing literature, various machine learning methodologies have been developed -like Bayesian network, Recurrent Neural Network and others - that can address intrinsic issues (eg. time-dependencies). Among the machine learning models commonly utilized for predicting mycotoxins in literature, the Bayesian Network model stands out that combines expert knowledge with statistical methods, making it a powerful tool for predictions.

A study [115] compared the predictive performance of the traditional models (mechanistic and empirical) with the machine learning approach (Bayesian Network) for DON contamination in wheat samples using the same input datasets (weather data, agronomic data, and individual DON content of 625 wheat samples (2001-2013)). According to the results of the study [115], the predictive performance of empirical model (88%) found higher than the mechanistic model (80%) while the accuracy of machine learning approach was 86%, the machine learning approach exhibited the advantage of ease of implementation with limited input data. Although the Bayesian Network model is often used [115,117,118] to demonstrate the interaction between the food safety hazards and the influential factors with high accuracy (>86%), research has indicated room for enhancing the model with supplementary data sources [117,118].

The neural network framework is designed as a graphical representation with nodes (neurons) and edges (connections between neurons). Each neuron processes input from the aggregated outputs of neurons linked via input edges. Collectively, these neurons engage in intricate computation [176], with outputs determined by an amalgamation of nonlinear input variables and middleware values. The recurrent neural network model discerns time-dependencies [15] and related non-linearities [16] bolstering the model's predictive prowess.

While comprehending the model's decision making process can be challenging, one study effectively employed a neural network to predict DON in wheat based on parameters like

previous crop, soil cultivation, and meteorological data (such as daily average temperature, total precipitation, and relative humidity), achieving 87% accuracy [177]. Interestingly, only one machine learning model was developed to predict the risk factor of AF presence in dried figs in the Southern Peloponnese based on soil parameters [17]. No predictive study has been conducted considering the effect of meteorological parameters based on the time-dependencies and linear and non-linear dependencies on AF contamination in dried figs in Turkey. Therefore, the main objective of this study was to develop predictive modeling for AF contamination in Turkish dried figs (using the total number of notification data in the RASFF database) based on meteorological parameters using a machine learning approach which is RNN. This models aims to identify AF contamination risks prior to processing (before pre-harvest) and provide insights into contamination trends that can be used for the development of risk control strategies to minimize the mycotoxin contamination in Turkish dried figs.

## 4.2. METHODS

### 4.2.1. Database

#### 4.2.1.1. Climate Data

Weather-related data for the years 2002-2020 including monthly mean temperature, maximum temperature, the number of days when the temperature was higher than 30°C, total precipitation (mm), mean humidity (%), maximum humidity (%), and the number of rainy days were collected from the Turkish General Directorate of Meteorology for the main fig producing and exporting regions of Aydın (Bozdoğan, Buharkent, Germencik, İncirliova/İncir Araştırma Enstitüsü, Köşk, Kuyucak, Kuyucak/Musakolu Köyü, Nazilli, Söke, Söke/Karakaya Köyü) and İzmir (Beydağ, Kiraz, Ödemiş, Ödemiş/Demirdere Köyü, Selçuk, Sultanhisar, Tire, Torbalı) (except for Efeler and Yenipazar, Aydın) provinces [93].

#### 4.2.1.2. Export Data

Monthly export data for Turkish dried figs were obtained from the Turkish Statistical Institute (TUIK) for 19 RASFF members (France, Netherlands, Germany, Italy, Denmark, Greece, Portugal, Spain, Belgium, Luxemburg, Norway, Sweden, Finland, Austria, Malta,

Czech Republic, Slovakia, Romania, and Bulgaria) for the investigated production years [130].

#### 4.2.1.3. *RASFF Data*

The total number of notifications data for the AF hazard of Turkish dried figs were obtained from the RASFF Portal [73] by selecting the origin of the product as “Turkey” and the hazard category as “mycotoxins”. The subject of notifications was restricted using the keyword “fig”.

A “production year” was defined as the 12-month period starting in mid-September and ending mid-September of the following year for analysis purposes considering the production season of dried figs, which starts in the middle of September. A total of 1052 notifications (including 967 dried figs, 33 dried fig pastes, 31 organic dried figs, 18 diced dried figs, and 3 boiled dried figs) for mycotoxin hazard of Turkish dried figs reported between 15/09/2002-15/09/2020 was included in the analysis. The RASFF notification data was normalized using export data (obtained from TUIK) to remove the effect of export quantities.

### 4.2.2. **Statistical Analysis**

#### 4.2.2.1. *Month-Based Models*

Multiple linear regression analysis was carried out using Python to develop a model for the prediction of the dependent variable (monthly number of AF related notifications for Turkish dried figs for the years 2002-2020) from the independent variables (7 monthly meteorological variables including number of rainy days, maximum relative humidity, average relative humidity, number of days with maximum temperature above 30°C, maximum temperature, average temperature, and total precipitation).

To address the temporal relationship between input (meteorological data) and output (total number of notification) data, 12 different models with increasing number of monthly lags were tested over years. The first model predicted the monthly RASFF data from the meteorological data for the same month. The second model predicted the monthly RASFF notification data from the meteorological data obtained in the previous month. For example,

the research question was “Did the meteorological data observed in September affect the number of RASFF notifications in October for the prediction of RASFF notifications”. The third model predicted monthly RASFF notification data from the meteorological data obtained two months before. This pattern iteratively continued for 11 months resulting in 12 models in total.

#### 4.2.2.2. *Season-Based Models*

Ridge regression as a variant of multiple linear regression was carried out to predict RASFF notification data from seasonal meteorological data. The relationship between 7 meteorological factors during the growth season of figs (January-September) and the sum of total number of notifications obtained in October, November, December, and January were thus assessed. Considering that this resulted in the number of independent variables being greater than the data samples, the models were regularized by penalizing the L2 norm to prevent overfitting.

Cross-validation was used to predict the total number of notifications between October and January using the (input) meteorological variables for the months January-September (resulting in 9 months x 7 meteorological parameters = 63 input variables). It involved modeling the data (fitting the data to the model) 20 times by leaving out a different separate year in each fold, and using the left-out year for the evaluation of the fitted model. Therefore 20 models were created, and each model yielded one prediction, which was obtained using the year left out for the modeling. The actual and predicted values were then correlated to quantify performance of the model (with Pearson’s  $r$  and corresponding Student’s  $t$  test  $p$  value). The regression coefficients obtained in each of the 20 folds were averaged (Figure 4.1 (a)) and their standard deviations were calculated (Figure 4.1 (b)) to get an indication of the contribution of individual parameters.

In the next stage, the model was fitted separately for October, November, December, and January (rather than the October-January data as sum) with cross-validation. The correlation between the actual and predicted values, and the  $t$ -test was carried out for the four models generated as described above. The average regression coefficients and their standard deviations are presented in Figures 4.2 (a) and 4.2 (b), respectively.

#### 4.2.2.3. *Time Series Analysis*

Recurrent neural networks (RNN) were used to develop a time-series model to account for time-dependencies and non-linearities in the data and improve predictive performance of the model. The model was used to predict the dependent variable (monthly number of notifications) and to understand the temporal dependencies between the independent variables (7 monthly meteorological factors) and the dependent variable. Specifically, PyTorch was used to build a gated recurrent unit (GRU) architecture with 100 hidden units to capture long-term dependencies in data by selectively updating its hidden state based on each input, followed by a linear output layer for prediction.

Time series cross-validation that maintains the temporal order of the data was used to evaluate the model's generalization performance on new data. The data was split into five folds using the TimeSeriesSplit function from scikit-learn. In each fold, the model was trained on the training set and its predictions over the validation set were aggregated. The mean squared error loss and Adam optimizer were used for 1000 epochs in each fold. Adam is an optimization algorithm for stochastic gradient descent (an iterative method for optimizing an objective function with suitable smoothness properties) for training deep learning models.

After training the model using time series cross-validation, the performance of the model was evaluated by computing the Pearson correlation coefficient between the aggregated predictions and the ground truth values of the dependent variable. The prediction could not be carried out for the first 32 months because there was no corresponding past data to train the model.

To calculate the importance (positive or negative contribution) of each feature (meteorological parameter at a given time), a custom function that computed the gradients of the output with respect to the inputs using backpropagation was implemented. The function takes the trained model and input data as inputs and returns an importance variable containing the importance scores for each input feature. The function was repeated for each output time step. For each output, its gradients with respect to each past input feature were stored in the importance variable. The importance variable represented the importance scores of any feature at any time step for any future output, which were then visualized and analyzed to gain insights into the temporal dependencies between the independent variables and the

dependent variable (Table 4.6). The Table 4.6 demonstrated the contribution quantity of meteorological parameters with units when a number of notification is increased or decreased. This enabled the analysis of the contribution of any number of selected meteorological parameter(s) (eg. monthly average temperature and relative humidity) observed at a selected time frame (eg. July-August 2017) on the total number of notifications obtained at a selected time point (eg. September 2018).

### 4.3. RESULTS AND DISCUSSION

#### 4.3.1. Month-Based Models

Month-based models using multiple linear regression were not able to predict total number of AF related RASSF notifications for Turkish dried figs from 7 meteorological variables (Table 4.1). This means that the meteorological data of a given month was not able to predict the notification data obtained for the same month, for the previous month, for two months before, up until 11 months. The inability of month-based models to predict monthly number of notifications is expected as the main assumption of these models – that monthly notification data can be predicted by a weather data (including 7 meteorological parameters) of a single month – does not take into account the continuous nature of the effect. Yet month-based modeling was carried out to provide a baseline for further predictive modeling of the data.

Table 4. 1. Multiple linear regression analysis results (Month-Based Models)

|     | <b>r</b> | <b>p</b> |
|-----|----------|----------|
| x   | 0.11     | 0.13     |
| x-1 | -0.10    | 0.18     |
| x-2 | 0.07     | 0.33     |
| x-3 | -0.08    | 0.27     |
| x-4 | 0.04     | 0.58     |
| x-5 | -0.11    | 0.12     |
| x-6 | 0.07     | 0.33     |

|      |       |      |
|------|-------|------|
| x-7  | -0.08 | 0.27 |
| x-8  | 0.07  | 0.37 |
| x-9  | -0.13 | 0.09 |
| x-10 | 0.05  | 0.49 |
| x-11 | -0.05 | 0.52 |

\*x: same month, x-1: previous month,  
x-2: two months before, and until 11 months,  
not corrected for multiple comparisons

#### 4.3.2. Season-Based Models

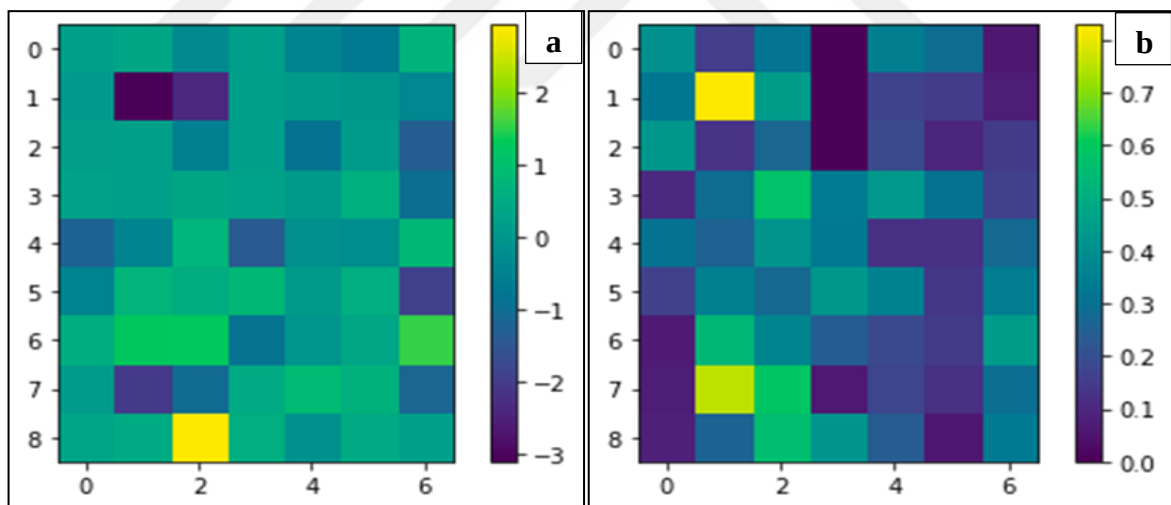
Season-based models with ridge regression was used to address the main limitation of the month-based models by using meteorological parameters throughout the growing season (Oct-Jan) for the prediction of notification data obtained during export season. The developed season-based model predicted the sum of the total number of RASFF notifications from October to January using seasonal meteorological data (from January to September) with an  $r$  value of 0.68 and  $p < 0.05$ .

Figure 4.1 (a) shows the average of the regression coefficients over 20 models and Figure 4.1 (b) shows their standard deviation. In both figures, the x-axes indicate the meteorological parameters, the y-axes indicate the months for growing season. Figure 4.1 (a) provides a measure of the individual contribution of each meteorological parameter, while Figure 4.1 (b) provides a measure of the consistency of the observed contribution. Color coding in Figure 4.1 (a) was done using the z-scored weights of the independent variables (from approximately -3 to 3) (Table 4.2). A positive weight indicates positive contribution to the dependent variable (i.e. an increase in the dependent variable (the number of AF-related notifications for Turkish dried figs) with an increase in the independent variable (meteorological parameter)), and a negative weight indicates a negative contribution. The absolute value of the weight provides an estimate of the magnitude of the contribution (Table 4.2).

All the meteorological parameters did not contribute to the predictive power of the model to the same extent (Figure 4.1 (a)). The parameters with large contributions were maximum

relative humidity (x-axis: 1), average relative humidity (x-axis: 2) and total precipitation (x-axis: 6). The positive correlation between rainfall and relative humidity should be noted while interpreting the results [178].

Maximum relative humidity (x-axis: 1) in February (y-axis:1) and August (y-axis:7) had a large negative contribution ( $-3.11\pm0.83$ ,  $-2.03\pm0.76$ , respectively). Interestingly, the nature of the contribution of average relative humidity (x-axis: 2) was dependent on the month. While average relative humidity (x-axis: 2) in February (y-axis:1) had a large negative contribution ( $-2.37\pm0.45$ ) to the sum of total number of notifications observed in export season (October, November, December, and January) (i.e. decreased the sum of the total number of notifications), in September (y-axis:8) it had a large positive contribution ( $2.95\pm0.56$ ) (i.e. increased the total number of notifications). Total precipitation (x-axis:6) in June (y-axis: 5) had a large negative contribution ( $-1.88\pm0.35$ ) to the sum of total number of notifications observed in the export season (October, November, December, and January) (i.e. decreased the sum of the total number of notifications).



\*y axis: growth season month (starting from January (0) until September (8), x axis: meteorological variables where 0 is the number of rainy days per month, where 1 is the maximum monthly relative humidity, where 2 is the monthly average relative humidity, where 3 is the number of days when the maximum air temperature is above 30°C in a month, where 4 is the maximum monthly temperature, where 5 is the average monthly temperature, where 6 is the monthly total precipitation

Figure 4. 1. The effect of meteorological parameters on the total number of notifications (October-January) with the average the regression coefficients (a) and standard deviation of the regression coefficients (b)

Table 4. 2. Regression coefficients over 20 models (mean  $\pm$  standard deviation)

| Months | Meteorological Parameters |                  |                  |                  |                  |                  |                  |
|--------|---------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|        | 0                         | 1                | 2                | 3                | 4                | 5                | 6                |
| 0      | 0.24 $\pm$ 0.41           | 0.38 $\pm$ 0.16  | -0.28 $\pm$ 0.32 | 0.25 $\pm$ 0.00  | -0.43 $\pm$ 0.35 | -0.68 $\pm$ 0.29 | 0.72 $\pm$ 0.06  |
| 1      | 0.07 $\pm$ 0.32           | -3.11 $\pm$ 0.83 | -2.37 $\pm$ 0.45 | 0.25 $\pm$ 0.00  | 0.10 $\pm$ 0.17  | 0.00 $\pm$ 0.15  | -0.35 $\pm$ 0.07 |
| 2      | 0.20 $\pm$ 0.42           | 0.25 $\pm$ 0.13  | -0.53 $\pm$ 0.27 | 0.25 $\pm$ 0.00  | -0.85 $\pm$ 0.19 | 0.12 $\pm$ 0.09  | -1.29 $\pm$ 0.15 |
| 3      | 0.29 $\pm$ 0.11           | 0.25 $\pm$ 0.29  | 0.37 $\pm$ 0.58  | 0.30 $\pm$ 0.33  | 0.09 $\pm$ 0.43  | 0.62 $\pm$ 0.31  | -0.94 $\pm$ 0.17 |
| 4      | -1.18 $\pm$ 0.31          | -0.40 $\pm$ 0.26 | 0.75 $\pm$ 0.42  | -1.38 $\pm$ 0.33 | -0.13 $\pm$ 0.13 | -0.19 $\pm$ 0.13 | 0.84 $\pm$ 0.28  |
| 5      | -0.44 $\pm$ 0.17          | 0.73 $\pm$ 0.35  | 0.58 $\pm$ 0.28  | 0.82 $\pm$ 0.43  | 0.09 $\pm$ 0.36  | 0.60 $\pm$ 0.14  | -1.88 $\pm$ 0.35 |
| 6      | 0.57 $\pm$ 0.07           | 1.27 $\pm$ 0.54  | 1.30 $\pm$ 0.37  | -0.82 $\pm$ 0.25 | -0.03 $\pm$ 0.19 | 0.40 $\pm$ 0.15  | 1.57 $\pm$ 0.45  |
| 7      | 0.12 $\pm$ 0.07           | -2.02 $\pm$ 0.76 | -1.02 $\pm$ 0.59 | 0.51 $\pm$ 0.06  | 0.90 $\pm$ 0.18  | 0.66 $\pm$ 0.12  | -1.12 $\pm$ 0.30 |
| 8      | 0.33 $\pm$ 0.08           | 0.54 $\pm$ 0.26  | 2.95 $\pm$ 0.56  | 0.59 $\pm$ 0.42  | -0.14 $\pm$ 0.24 | 0.46 $\pm$ 0.06  | 0.24 $\pm$ 0.33  |

\*Months: Growth season month (starting from January (0) until September (8), meteorological variables where 0 is the number of rainy days per month, where 1 is the maximum monthly relative humidity, where 2 is the monthly average relative humidity, where 3 is the number of days when the maximum air temperature is above 30°C in a month, where 4 is the maximum monthly temperature, where 5 is the average monthly temperature, where 6 is the monthly total precipitation

The season-based model that was fitted separately for October, November, December, and January (rather than the October-January data as a sum), was able to significantly predict the RASFF notifications only in December ( $r=0.67$ ,  $p<0.05$ ) and January ( $r=0.58$ ,  $p<0.05$ ) using seasonal meteorological data.

Figure 4.2 (a) and Figure 4.2 (c) show the average of the regression coefficients over 20 models in December and January and Figures 4.2 (b) and (d) show the standard deviation of regression coefficients in December and January, respectively. In all figures, while the x-axis demonstrated the meteorological parameters, the y-axis demonstrated the months for growing season like in Figure 4.1. Figure 4.2 (a) and 4.2 (c) provide a measure of the individual contribution of each meteorological parameter, while Figure 4.2 (b) and 4.2 (d) provide the consistency of the observed contribution. Color coding in Figure 4.2 (a) was done using the z-scored weights of the independent variables (from approximately -4 to 2), while in Figure 4.2 (c) was done using the z-scored weights of the independent variables (from approximately -3 to 3) (Table 4.2). As indicated above, a positive weight indicates a positive contribution to the dependent variable (i.e. an increase in the dependent variable

(the number of AF-related notifications for Turkish dried figs) with an increase in the independent variable (meteorological parameter)), and a negative weight indicates a negative contribution. The absolute value of the weight provides an estimate of the magnitude of the contribution (Table 4.3 for December and Table 4.4 for January).

All the meteorological parameters did not contribute to the predictive power of the model to the same extent for December (Figure 4.2 (a)) and for January (Figure 4.2 (c)). The parameters with notable contributions are listed in Table 4.5.

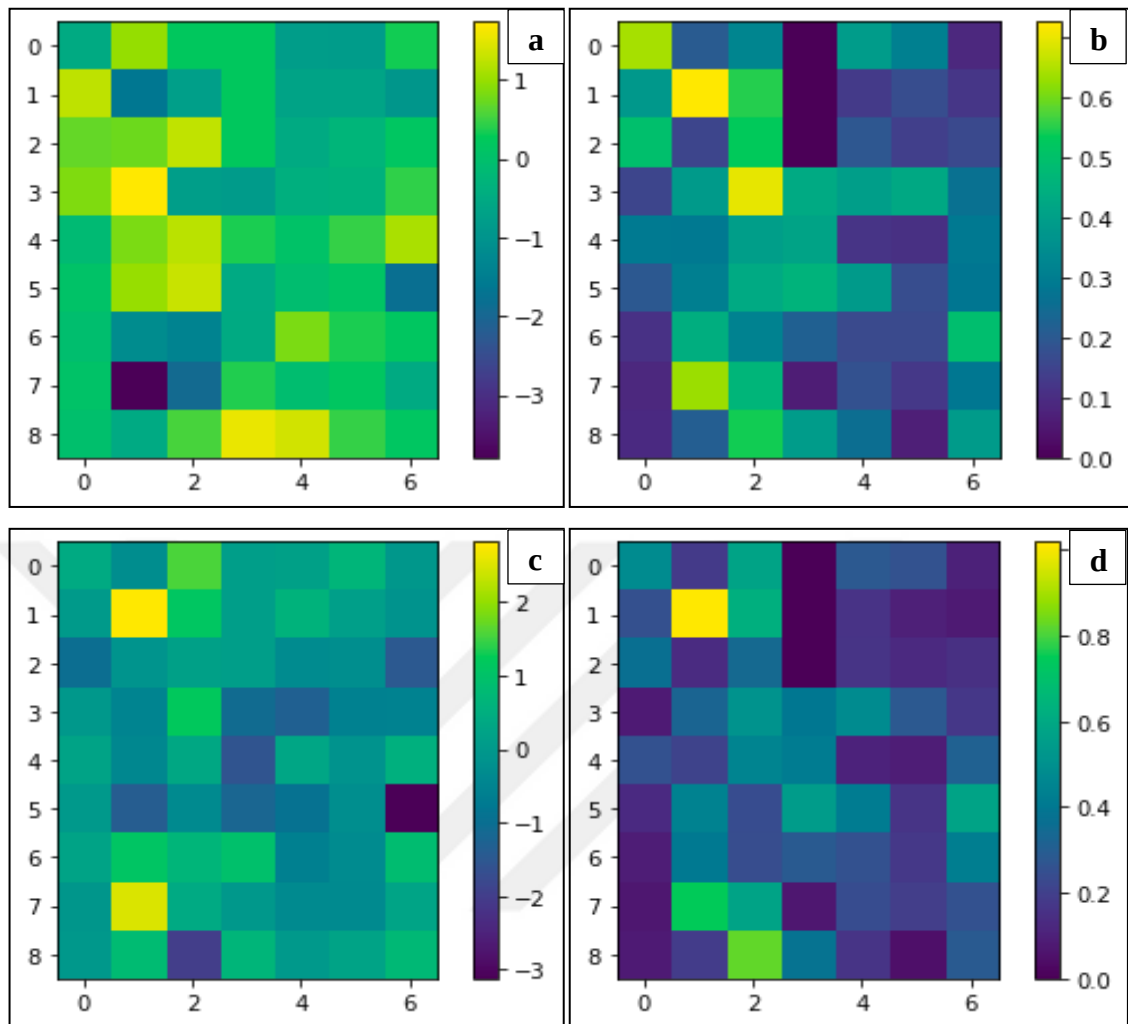
Similar to the previous results, maximum relative humidity (x-axis: 1), average relative humidity (x-axis: 2) and total precipitation (x-axis:6) were the meteorological parameters with the largest contribution to the total number of notifications in December and January (Figure 4.2 (a) and Figure 4.2 (c)). Also, differently ‘the number of days when the maximum air temperature is above 30°C had a large contribution to the prediction of the total number of notifications in December (Figure 4.2 (a)).

Interestingly, the maximum relative humidity (x-axis:1) in February (y-axis:1) and August (y-axis:7) had opposing effects in December and January ( $-1.63 \pm 0.73$ ,  $-3.79 \pm 0.63$ , and  $2.82 \pm 1.01$ ,  $2.50 \pm 0.75$ , respectively) while the maximum relative humidity (x-axis:1) observed in April (y-axis:3) had a large positive contribution on the number of notifications only in December ( $1.73 \pm 0.38$ ). Also, the average relative humidity (x-axis:2) had a negative contribution both in December and January ( $-1.91 \pm 0.46$ ,  $-1.99 \pm 0.83$ ), yet the effect was evident for different months (August and September, respectively). The contribution of total precipitation in June was similar for both months (i.e. a large negative contribution -  $1.79 \pm 0.28$  and  $-3.12 \pm 0.58$ , respectively). The number of days when the maximum air temperature is above 30°C (x-axis:3) in September (y-axis: 8) had large (positive) contribution only in December ( $1.54 \pm 0.39$ ) (Figure 4.2 (a)).

Our results showed the effects of a number of relative humidity and related parameters (maximum and average relative humidity, and total precipitation) on total number of notifications in December and January. Yet the observed temperature effect was limited to a single parameter (i.e. a positive contribution for the number of days when the maximum air temperature is above 30°C in September). While average relative humidity in August and September negatively affected the total number of notifications (contamination) in December and January, respectively, the effect maximum relative humidity was dependent

on the month. The maximum relative humidity in February and August affected the total number of notifications in December and January negatively and positively, respectively. In addition, total precipitation in June negatively affected the total number of notifications in December, and January.

While these results provide some insight into the effect of individual meteorological parameters (on a monthly basis) on the notification data (hence on AF contamination of Turkish dried figs), they fail to take into consideration time-dependencies and non-linearities in the data due to limitation of the method used. Therefore no attempt is made here to interpret the findings. The contribution of each meteorological parameter to the total number of notification is discussed in the next section based on the results of time series analysis using RNN.



\*y axis: growth season month (starting from January (0) until September (8), x axis: meteorological variables where 0 is the number of rainy days per month, where 1 is the maximum monthly relative humidity, where 2 is the monthly average relative humidity, where 3 is the number of days when the maximum air temperature is above 30°C in a month, where 4 is the maximum monthly temperature, where 5 is the average monthly temperature, where 6 is the monthly total precipitation

Figure 4. 2. The effect of meteorological parameters on the number of notifications obtained in December and January with the average the regression coefficients (a and c, respectively) and standard deviation of the regression coefficients (b and d, respectively)

Table 4. 3. The average of the regression coefficients with the standard deviation of regression coefficients (December)

| Meteorological Parameters |            |           |           |           |            |            |           |
|---------------------------|------------|-----------|-----------|-----------|------------|------------|-----------|
| Months                    | 0          | 1         | 2         | 3         | 4          | 5          | 6         |
| 0                         | -0.50±0.64 | 0.99±0.21 | 0.21±0.32 | 0.22±0.00 | -0.78±0.39 | -0.82±0.31 | 0.34±0.09 |

|   |            |            |            |            |            |            |            |
|---|------------|------------|------------|------------|------------|------------|------------|
| 1 | 1.25±0.38  | -1.63±0.73 | -0.74±0.55 | 0.22±0.00  | -0.66±0.13 | -0.63±0.17 | -0.94±0.12 |
| 2 | 0.71±0.50  | 0.75±0.16  | 1.27±0.53  | 0.22±0.00  | -0.50±0.20 | -0.24±0.14 | 0.16±0.16  |
| 3 | 0.85±0.15  | 1.73±0.38  | -0.76±0.70 | -0.84±0.43 | -0.39±0.40 | -0.35±0.43 | 0.45±0.27  |
| 4 | -0.13±0.29 | 0.83±0.29  | 1.23±0.40  | 0.36±0.41  | 0.08±0.12  | 0.46±0.11  | 1.12±0.29  |
| 5 | 0.09±0.20  | 1.01±0.31  | 1.30±0.43  | -0.52±0.46 | -0.08±0.38 | 0.12±0.18  | -1.80±0.28 |
| 6 | -0.02±0.11 | -1.15±0.44 | -1.37±0.32 | -0.47±0.22 | 0.83±0.17  | 0.36±0.17  | 0.19±0.50  |
| 7 | 0.08±0.09  | -3.79±0.63 | -1.91±0.46 | 0.39±0.06  | -0.07±0.18 | 0.19±0.13  | -0.51±0.29 |
| 8 | 0.00±0.09  | -0.53±0.22 | 0.56±0.54  | 1.54±0.39  | 1.38±0.26  | 0.46±0.07  | 0.17±0.39  |

\*Months: Growth season month (starting from January (0) until September (8), meteorological variables where 0 is the number of rainy days per month, where 1 is the maximum monthly relative humidity, where 2 is the monthly average relative humidity, where 3 is the number of days when the maximum air temperature is above 30°C in a month, where 4 is the maximum monthly temperature, where 5 is the average monthly temperature, where 6 is the monthly total precipitation

Table 4. 4. The average of the regression coefficients with the standard deviation of regression coefficients (January)

| Meteorological Parameters |            |            |            |            |            |            |            |
|---------------------------|------------|------------|------------|------------|------------|------------|------------|
| Months                    | 0          | 1          | 2          | 3          | 4          | 5          | 6          |
| 0                         | 0.44±0.48  | -0.24±0.18 | 1.56±0.57  | 0.14±0.00  | 0.18±0.28  | 0.69±0.25  | -0.04±0.10 |
| 1                         | 0.01±0.25  | 2.82±1.01  | 1.13±0.62  | 0.14±0.00  | 0.59±0.16  | 0.17±0.10  | -0.14±0.07 |
| 2                         | -0.99±0.36 | -0.11±0.13 | 0.18±0.34  | 0.14±0.00  | -0.31±0.16 | -0.24±0.12 | -1.45±0.15 |
| 3                         | -0.02±0.08 | -0.48±0.32 | 1.20±0.51  | -1.07±0.40 | -1.29±0.48 | -0.55±0.28 | -0.54±0.17 |
| 4                         | 0.24±0.25  | -0.40±0.21 | 0.34±0.45  | -1.57±0.42 | 0.31±0.10  | -0.14±0.09 | 0.55±0.31  |
| 5                         | -0.00±0.13 | -1.36±0.44 | -0.34±0.24 | -1.18±0.54 | -0.90±0.41 | -0.22±0.16 | -3.12±0.57 |
| 6                         | 0.25±0.09  | 1.10±0.40  | 0.70±0.24  | 0.96±0.28  | -0.58±0.25 | -0.31±0.17 | 0.85±0.42  |
| 7                         | -0.07±0.07 | 2.50±0.74  | 0.38±0.57  | -0.03±0.07 | -0.34±0.23 | -0.34±0.19 | 0.27±0.25  |
| 8                         | -0.02±0.08 | 0.84±0.19  | -1.98±0.82 | 0.67±0.38  | -0.00±0.16 | 0.25±0.04  | 0.77±0.28  |

\*Months: Growth season month (starting from January (0) until September (8), meteorological variables where 0 is the number of rainy days per month, where 1 is the maximum monthly relative humidity, where 2 is the monthly average relative humidity, where 3 is the number of days when the maximum air temperature is above 30°C in a month, where 4 is the maximum monthly temperature, where 5 is the average monthly temperature, where 6 is the monthly total precipitation

Table 4. 5. Effects of monthly meteorological parameters on the prediction of the total number of notifications in December and January (Season-Based Models)

| <b>Meteorological Parameters</b>                                 | <b>Month</b> | <b>Effect (+/-)</b> |
|------------------------------------------------------------------|--------------|---------------------|
| <i>Total number of notifications in December</i>                 |              |                     |
| Maximum relative humidity                                        | February     | -1.63±0.73          |
| Maximum relative humidity                                        | August       | -3.79±0.63          |
| Maximum relative humidity                                        | April        | +1.73±0.38          |
| Average relative humidity                                        | August       | -1.91±0.46          |
| Total precipitation                                              | June         | -1.80±0.28          |
| The number of days when the maximum air temperature is above 30° | September    | +0.67±0.38          |
| <i>Total number of notifications in January</i>                  |              |                     |
| Maximum relative humidity                                        | February     | +2.82±1.01          |
| Maximum relative humidity                                        | August       | +2.50±0.74          |
| Average relative humidity                                        | September    | -1.98±0.82          |
| Total precipitation                                              | June         | -3.12±0.57          |

#### 4.3.3. Time Series Analysis

Time series analysis was performed to improve predictive performance of the model by addressing time-dependencies and non-linearities in the data. The calculation of the importance score also enabled the investigation of the contribution of each meteorological parameter to the total number of notifications. The developed time series model predicted the total number of RASFF notifications using monthly meteorological data with a  $r$  value of 0.64 and  $p < 0.05$ . The actual and predicted number of RASFF notifications are presented in Figure 4.3.

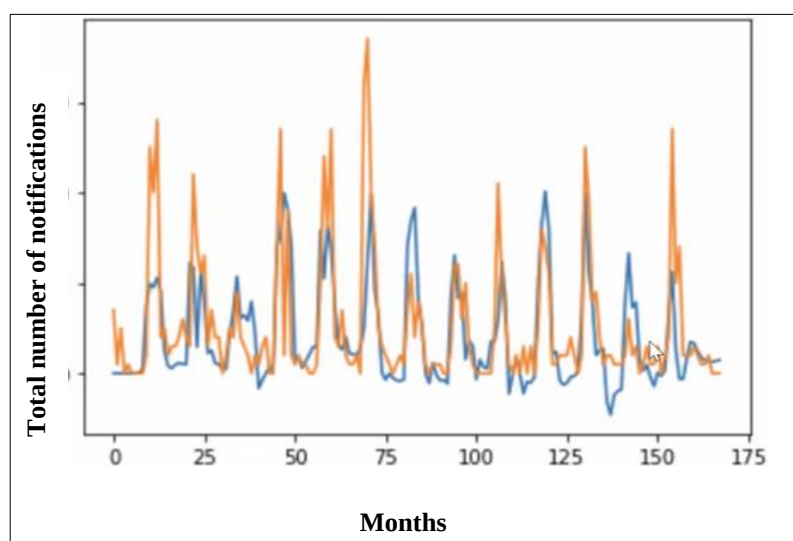


Figure 4. 3. The notified (RASFF, orange) and predicted (developed model, blue) total number of notifications using time series model

The effect of monthly meteorological parameters on the total number of notifications in the export period (in October - January) were investigated using the importance scores (calculated as described in Section 4.2.2.3 as the change in the total number of notifications with one unit change in the meteorological parameter). An importance score of  $\pm 0.30$  (corresponding to  $\pm 3.3$  units change in the meteorological parameter per one unit change in total number of notifications) was chosen as a threshold for the interpretation of the effects (Table 4.6.).

Table 4. 6. Importance scores for monthly meteorological parameters showing their effect on the total number of notifications in the export period of dried figs (October-January)

| Meteorological Parameter<br>(unit of measurement)                           | Month  | Importance<br>Score* | 1/ Importance Score**<br>(Units Change of<br>Meteorological<br>Parameter/ One Unit<br>Change of Number of<br>Notifications) |
|-----------------------------------------------------------------------------|--------|----------------------|-----------------------------------------------------------------------------------------------------------------------------|
| The number of days when the maximum<br>air temperature is above 30°C (days) | June   | 0.52                 | +1.9                                                                                                                        |
| The number of days when the maximum<br>air temperature is above 30°C (days) | July   | 0.82                 | +1.2                                                                                                                        |
| The number of days when the maximum<br>air temperature is above 30°C (days) | August | 0.93                 | +1.1                                                                                                                        |

|                                                                          |           |      |      |
|--------------------------------------------------------------------------|-----------|------|------|
| The number of days when the maximum air temperature is above 30°C (days) | September | 0.80 | +1.3 |
| Maximum monthly temperature (°C)                                         | July      | 0.41 | +2.4 |
| Maximum monthly temperature (°C)                                         | August    | 0.38 | +2.6 |
| Average monthly temperature (°C)                                         | June      | 0.40 | +2.5 |
| Average monthly temperature (°C)                                         | July      | 0.56 | +1.8 |
| Average monthly temperature (°C)                                         | August    | 0.61 | +1.6 |
| Average monthly temperature (°C)                                         | September | 0.50 | +2.0 |

\* Importance score represents unit change in the meteorological parameter that causes the unit change in the number of notifications, \*\*1/Importance score represents the required units change of meteorological parameters for 1 unit change of number of notifications

The number of days when the maximum air temperature is above 30°C was the main meteorological parameter that contributed to the total number of notifications with importance scores of 0.52-0.93. Maximum and average monthly temperature in July and August, and average monthly temperature in June and September also had importance scores > 0.30 (0.38-0.61). Interestingly none of the humidity related parameters had an absolute importance score higher than 0.30.

The effects of meteorological parameters on mycotoxin contamination of food products have not been fully characterised in a time-dependent manner in the literature. Literature on the relationship between meteorological parameters and AF contamination in dried figs is rather limited. Bircan et al. (2008) [8] investigated AF contamination of Turkish dried figs (by analyzing dried fig samples collected from different export companies in Aydın province of Turkey during September and December of 2007) and used meteorological data (obtained from the Directorate of Aydın Meteorology) to interpret the effect of weather conditions. The substantial increase in AF contamination observed in 2007 compared to 2003-2006 [78] was attributed to drought stress, average high temperatures, and low average relative humidity experienced during the growing season of dried figs (January-September in 2007) [8]. When compared to 2003–2006, 2007 was characterized by higher average temperature (1–2°C), lower total precipitation (300 mm) and average relative humidity (10–15%). While monthly trends varied for total precipitation and average temperature, the mean average relative humidity was lower in each month of 2007 when compared with previous years [8]. Also, the average temperatures up to 30°C is desired especially during the fruit ripeness and drying period (July-September). While this situation is important for the sun-drying process,

higher temperatures ( $>40^{\circ}\text{C}$ ) cause damage to fruits, keeping them small and cause branch burning and affect aflatoxin contamination of dried figs mainly due to damage to the fig skin structure, cracking, and sunburned disease of the fig fruit [8,179]. Therefore, the all temperature related factors which contributed to the total number of notifications as the number of days when the maximum air temperature is above  $30^{\circ}\text{C}$ , the average and maximum temperature observed in the drying season can be related to the effect of drought conditions that causes the increase in contamination.

#### **4.4. CONCLUSION**

In conclusion, the developed time series model predicted the total number of RASFF notifications using monthly meteorological data with an r-value of 0.64. While the predictive accuracy of the model was similar to the season-based multiple regression method, the ability of the RNN model to account for non-linearity and time-dependencies in the data set resulted in a model with improved predictive capacity. The use of actual contamination data of Turkish dried figs obtained randomly (eg. from the Republic of Türkiye Ministry of Agriculture and Forestry control database) for modelling would result in further improvements in model performance and would enable a more rigorous assessment of effect of meteorological parameters. It should be noted that a more in-depth understanding of the effect of meteorological parameters on mycotoxin contamination requires further research involving statistical, field and experimental studies.

## 5. DESIGNING A WEB-BASED ANALYSIS AND FORECAST SYSTEM TO MINIMIZE AF RISK IN DRIED FIGS

### 5.1. INTRODUCTION

AFs are carcinogenic compounds that are produced by *Aspergillus spp.* under favorable conditions (i.e. 25-35°C, pH=6, and aw=0.98) [172]. AF contamination of dried figs, which might occur throughout the production chain, constitutes a major food safety risk with significant public health and economic consequences [180].

Design of analysis and forecast tools and systems to minimize food safety and related public health risks, and economic losses via providing up-to-date information for actors and decision-makers in the sector are gaining importance day by day in the face of rising challenges such as climate change [181]. Studies on predictive modeling of mycotoxin risk, which form the basis of these tools, have been reviewed in detail in Section 2.3.2. Prediction of AF risk from meteorological parameters (including temperature and humidity-related factors) offers an important avenue of research due to established relations with AF contamination and data availability.

Studies on the design and development of effective and user-friendly tools/systems the management of mycotoxin risk however have been limited. Two recent European Union projects on mycotoxin risk minimization (MycoKey and MyToolBox) include the development of systems/tools for the prediction of mycotoxin risk in maize and wheat, respectively in addition to integration of the expert knowledge. While the main aim of the MycoKey project was predicting global mycotoxin risk in maize and wheat [125], the aim of MyToolBox developed a predictive model to predict DON in wheat sample [124]. While the MycoKey project focused on the use of traditional mechanistic models such as AFLA-Maize [10] and FER-Maize [182] to predict AFB1 and fumonisins in maize, respectively. An updated, improved model developed by the integration of these two models was used as Tox\_Maize for the prediction of co-occurrence of mycotoxins [183], MyToolBox used Monte Carlo simulation models for pre-harvest control and post-harvest control in wheat and maize [184].

In the MycoKey project, models were improved by standardizing the effects of varying temperatures on the growth of *A. flavus* and *F. verticillioides*. Mycotoxin production data

was normalized with values ranging between 0 (no production) and 1 (maximum production). The growth of *A. flavus* and *F. verticillioides*, along with mycotoxin production as influenced by temperature, was assessed using non-linear regression analysis. This analysis utilized the Levenberg-Marquardt algorithm to minimize errors. Following this, Tox-Maize was developed based on a field study. Meteorological data - including temperature, relative humidity, and rainfall - were obtained hourly from stations close to the maize growing regions. Maize samples, collected from Italy in 2016 (25 samples) and 2017 (46 samples) were analyzed for AF and fumonisin content. The integrated model demonstrated a 20% reduction in the probability of maize contamination for fumonisin; however the results for AF contamination were not satisfactory.

Within the MyToolBox project, during the pre-harvest phase, the model estimated maize contamination levels at  $<5 \mu\text{g/kg}$ ,  $5\text{-}20 \mu\text{g/kg}$ , and  $>20 \mu\text{g/kg}$  AFB1. While BIOMIN provided the mycotoxin data confidentially for various European sub-regions, production data was sourced from Eurostat (2019). In the post-harvest phase, the model was used to determine the reduction in mycotoxin contamination when the legal limits are higher in maize samples. Data regarding the production of maize (from Europe) from 2009-2018 was available for analysis. The pre-harvest study revealed that if 80% of the farmers use one control measure, up to 9% of maize, having an AFB1 concentration of  $>20 \mu\text{g/kg}$ , would witness reduced contamination. This percentage rises to 17% for maize with a concentration of  $<5 \mu\text{g/kg}$ . Additionally, there is a single study on the development of a tool for the AF contamination of dried figs from soil parameters based on the location and altitude of the fig orchards located in the Southern Peloponnese [17].

Digital systems that monitor and forecast mycotoxin contamination risk can be effective in minimizing mycotoxin risk in dried figs by enabling/supporting risk management decisions and efficient use of resources. Therefore, this study aimed to design a web-based system that can be used by various actors in the dried fig supply chain to monitor and forecast AF risk in dried figs (from meteorological parameters based on the RNN model developed in Section 4).

The system includes two modules – Module 1 for public use, which is based on RASFF total notification data, and Module 2 for use by the Republic of Türkiye Ministry of Agriculture and Forestry, which is based on Ministry database on AF contamination data.

## 5.2. METHODS

### 5.2.1. Definition of the System

A web-based digital system (Mikotox) with two modules was designed to monitor weather-related risks of AF contamination and to forecast AF risk in Turkish dried figs based on RASFF database (Module 1: Mikotox-RASFF) and the Republic of Türkiye Ministry of Agriculture and Forestry mycotoxin contamination database (Module 2: Mikotox-AF).

#### *Predictive model and data*

The designed system (including Module 1 and Module 2) is based on the time-series model developed using RNN to predict AF risk of Turkish dried figs from meteorological parameters (Section 4). The input data for the model (seven meteorological parameters (monthly mean temperature, maximum temperature, the number of days when the temperature was higher than 30°C, total precipitation (mm), mean humidity (%), maximum humidity (%), and the number of rainy days) for the main fig producing and exporting regions (Aydın and İzmir)) was collected from the Turkish General Directorate of Meteorology. Total number of AF related notifications for Turkish dried figs obtained from the RASFF database was used as output data to characterise AF risk for modelling purposes considering data availability (as discussed in Section 4). While the prediction of RASFF related risks can be of value in itself to monitor and predict issues related to the export of Turkish dried figs, the use of RASFF notification data for the characterization of AF contamination risk limits the accuracy of the forecasting system. Thus, the developed system contained two modules to forecast RASFF related risks for public use (using RASFF notification data) and to forecast AF contamination risk of dried figs produced in Turkey for use by the Republic of Türkiye Ministry of Agriculture and Forestry (using contamination data collected by the Republic of Türkiye Ministry of Agriculture and Forestry).

#### *Module 1: Mikotox-RASSF*

Module 1 (Mikotox-RASFF) was designed for public use to forecast AF risk for Turkish dried figs (based on RASFF total notification data) from meteorological data for the fig-producing regions in Turkey. Analytical functionality was also incorporated into the system to monitor AF risk by the analysis of monthly and yearly notification trends, and to monitor

the effect of meteorological parameters on the total number of RASFF notifications. The effect of meteorological parameters was quantified based on the estimated importance scores (as described in Section 4). The effects are presented for the meteorological parameters and the time-frames (for the meteorological data and the total number of notifications) selected by the user.

#### *Module 2: Prediction of AF risks based on contamination data*

Module 2 (Mikotox-AFLATOXIN) was designed for use by the Republic of Türkiye Ministry of Agriculture and Forestry to forecast AF risk (based on Ministry database) from meteorological data for the fig-producing regions in Turkey. The system enables direct integration of the database into Module 2 via upload, and the utilization of the uploaded data for the training and validation of the model. Also, the system was designed to work dynamically. After the system obtaining online weather data and model will be updated. Additionally, similar to the Mikotox-RASFF module, analytical functionality was included to monitor the AF risk considering the analysis of monthly and yearly trends, and the effect of meteorological parameters on the total AF content. The effect of meteorological parameters was quantified using the estimated importance scores (as described in Section 4). The effects are presented for the meteorological parameters and the time-frames (for the meteorological data and the total AF content) selected by the user.

In order to improve the forecast accuracy of the model and to support research activities, the system enables integration of data (available to the Ministry) on other factors affecting AF contamination risk to the system. While selected production and processing related factors based on findings of this research (Section 3) are proposed, additional factors can be defined by the user.

### **5.3. RESULTS AND DISCUSSION**

#### **5.3.1. Description of The Main Risk (Monitoring and Forecasting) Functionalities**

##### *Monitoring AF risk*

Trends in the total number of RASFF notifications and trends in contamination data on a monthly and yearly basis were used to monitor AF risk in Module 1 and 2, respectively. The

monitoring function was thus based on the trends in data used for risk forecasting. Yearly and monthly RASFF notification trends have been effectively utilized both in the literature and in this thesis (Section 3) to study AF risk. The normalization of the notification data to remove effect of export quantities and inclusion of the other factors that might affect the number of notifications in the presentation (such as regulatory changes etc.) improves usefulness of RASFF data for the study of AF risk. Yearly trends provide important insights on the factors that might affect AF contamination, whereas monthly trends indicate the distribution of the notifications (with the highest values obtained in the main export season) (Section 3). The meteorological factors, which were used in Section 4 (and thus in the forecast function) for predictive modelling of AF risk in dried figs based on earlier research findings (Section 3), were included in the trend analysis to enable the study of their relationship with AF risk. The risk monitoring function also included the contribution of meteorological parameters to the AF risk (based on importance scores). In Module 1, this function enables the review of the total effect of meteorological parameters observed in the growth season on the total number of notifications in the main export period.

#### *Forecasting AF risk*

The forecasting functions in Modules 1 and 2 are based on the prediction of AF risk (quantified using RASFF notification data for Module 1 and Ministry AF concentration data for Module 2) using the RNN model (Section 4). As the RASFF portal only includes the data for notified samples (i.e. for samples which exceeds the maximum limit) the usefulness of RASFF concentration data for trend analysis and predictive risk modeling is rather limited. However, as the AF contamination (concentration data) in the Ministry database is obtained via random and regular sampling procedures (as outlined in Section 2.3.1) the use of raw concentration data in Module 2 is expected to improve prediction accuracy.

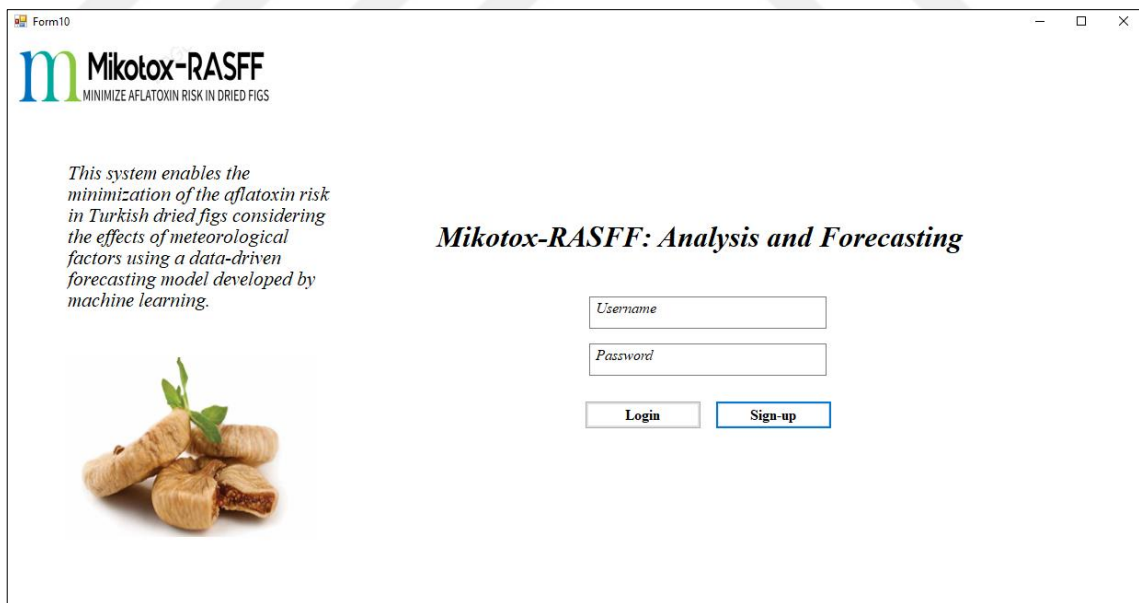
Based on the modeling results, the forecast function provides forecast values for monthly AF risk data for a 12-month time period in both modules. Alert thresholds were calculated based on statistical significance of AF risk data calculated using z-tables. This function was designed to enable the user to monitor risk forecast values continuously (eg. from the start of the growing season) and take preventive measures based on generated alerts. The forecast function also includes scenario-based forecasting, which calculates forecast values based on the change in selected meteorological parameters determined by the user. The added-on functionality for the collection of data for factors other than meteorological parameters is

based on earlier research in this thesis (Section 3) that suggest a correlation between several production and processing related parameters (such as harvest and drying method) and AF risk, which has not been quantified or statistically tested. The database of other factors generated in this manner will be included in modeling in the future to improve prediction accuracy, and can also be used for research purposes.

### 5.3.2. Description of the Modules

#### 5.3.2.1. Module 1: Mikotox-RASFF - Monitoring and Forecast of AF Risk (Based on RASFF Notification Data) in Dried Figs for Public Use

The homepage of the Module (Figure 5.1) includes a brief description of the system. In order to use the system, the user has to register by clicking the Sign-up button then can start to use the system using the username and password they selected (Figure 5.1). Information on the user (such as e-mail, user name, password, questionnaire to understand the user profile etc) is collected upon registration.



**Mikotox-RASFF**  
MINIMIZE AFLATOXIN RISK IN DRIED FIGS

*This system enables the minimization of the aflatoxin risk in Turkish dried figs considering the effects of meteorological factors using a data-driven forecasting model developed by machine learning.*

***Mikotox-RASFF: Analysis and Forecasting***

Username

Password

Figure 5. 1. Homepage of Module 1

Upon login, the module starts with a page that includes two buttons “Analysis”, and “Forecasting” (Figure 5.2), which enables the user to select one of the two main functionalities of the system.

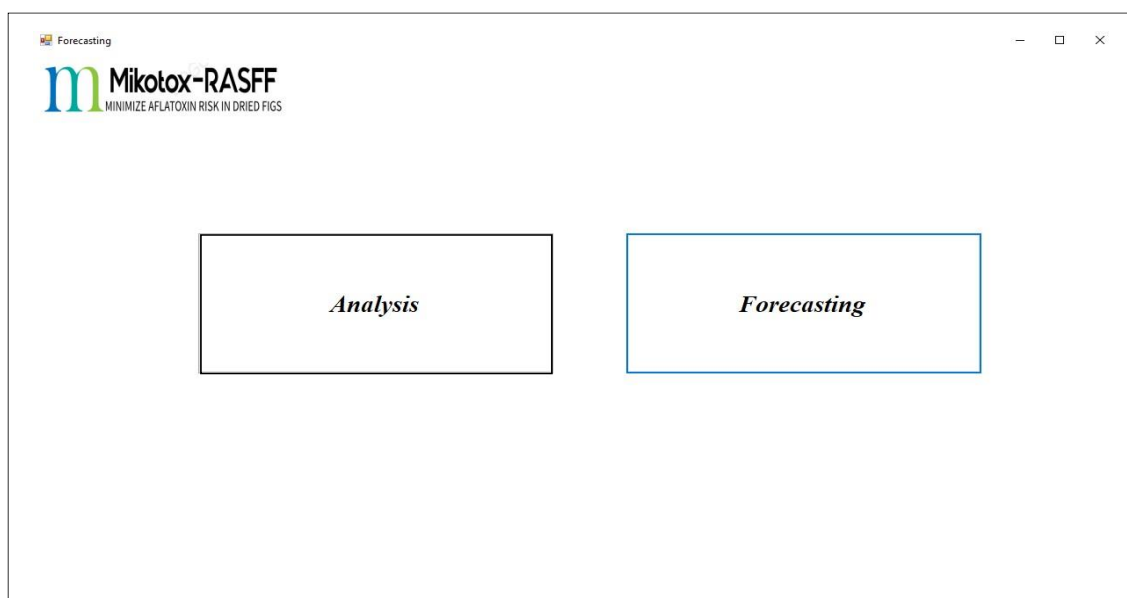


Figure 5. 2. Main page for Module 1 including two “Analysis” and “Forecasting” buttons

The user can access the analytical functionality by clicking on the “Analysis” button and by selecting the type of trend analysis (RASFF yearly trends, or RASFF monthly trends), on the upcoming page (Figure 5.3). The user can also access the monthly and yearly trends of meteorological factors and analysis of the contribution of meteorological factors based on importance scores as described in Section 4 under “Meteorological Parameters”.

If the user clicks the “By Year” button, RASFF yearly trends are presented using a bar graph showing the change in the total number of notifications by year (Figure 5.4). RASFF monthly trends are presented by a bar graph with two sliders that the user can use to select the month and year (Figure 5.5). This function is designed to enable the comparison of RASFF data based on the selected month and year to the user.

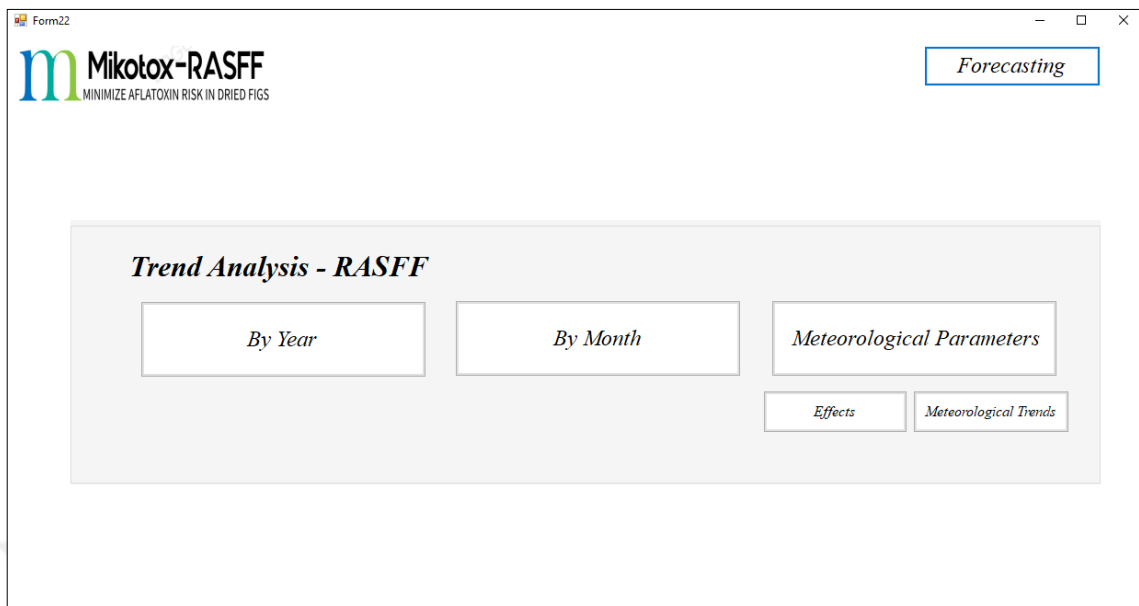


Figure 5. 3. Analysis of RASFF notifications page

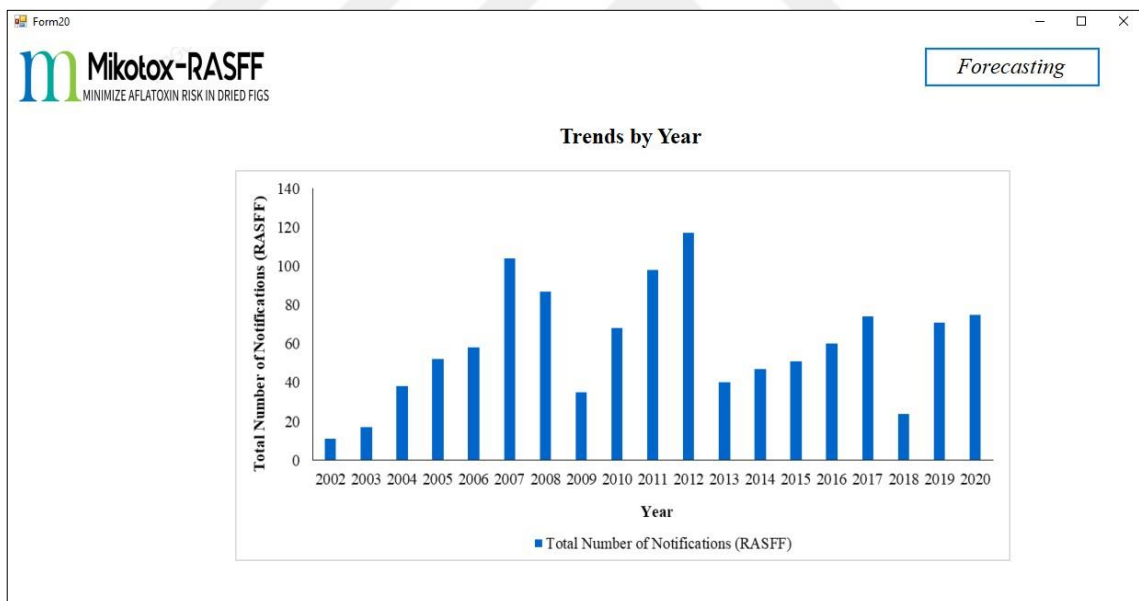


Figure 5. 4. Trends of RASFF data by year

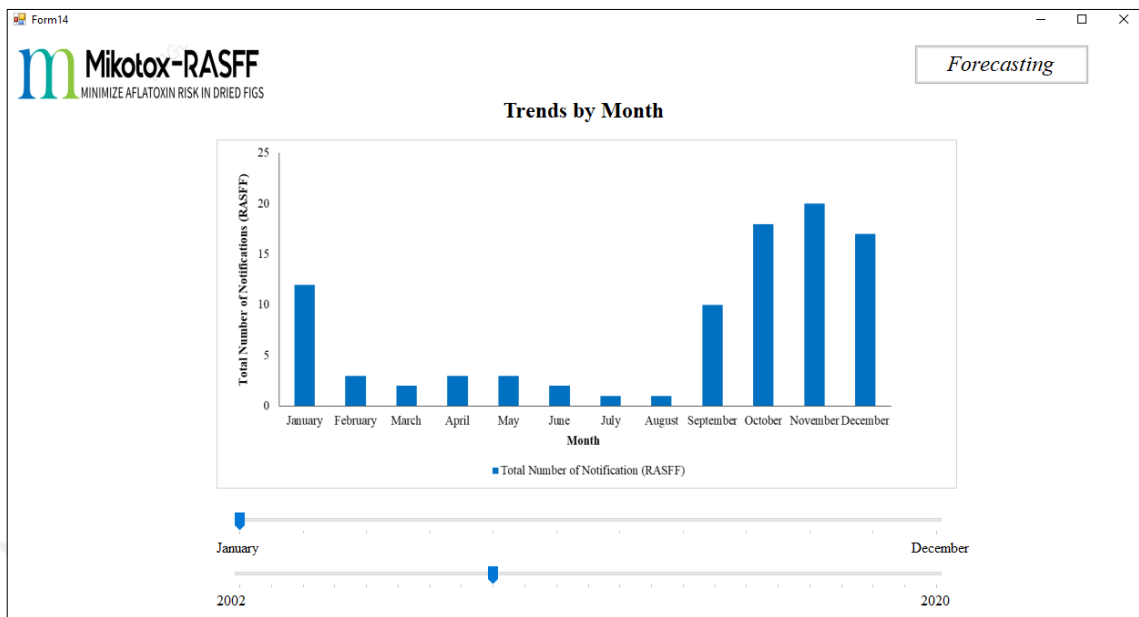


Figure 5. 5. Trends of RASFF data by a selected month and year

The meteorological trends for the selected parameters are integrated onto the bar graph showing the yearly/monthly trends (using line charts) when the user clicks the “Meteorological Trends” button on the yearly/monthly trend analysis page (Figure 5.6). When the user selects one or more of the meteorological parameters by clicking in the right part, he/she can see the trends in meteorological parameter(s) (monthly averaged) on the RASFF notifications as a line graph considering the selected month and years (slider).

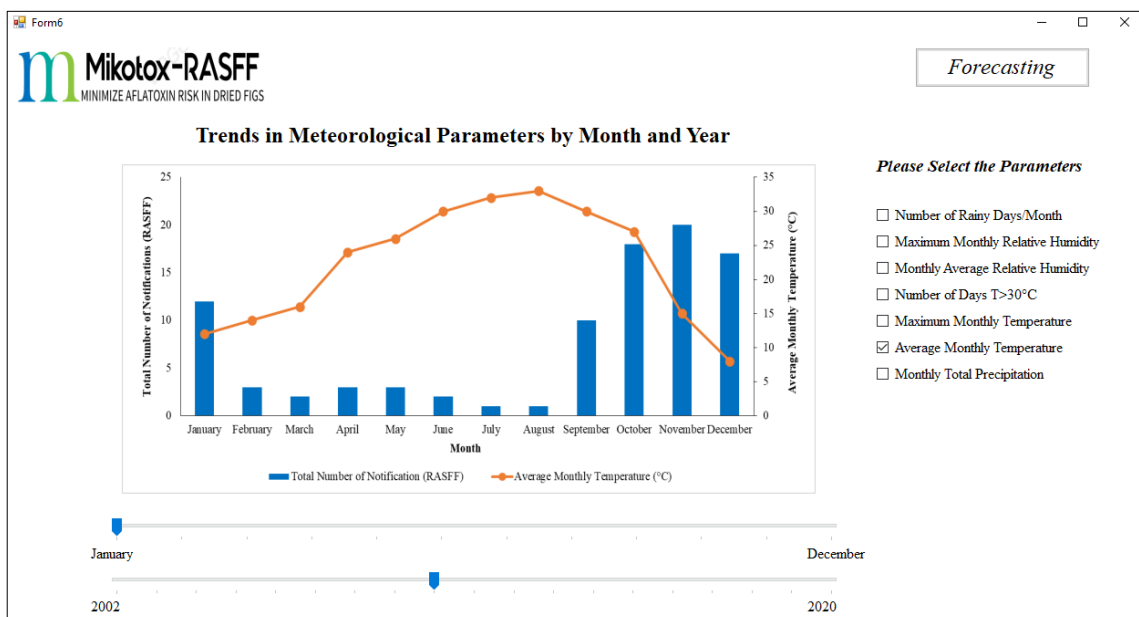


Figure 5. 6. Trends in meteorological data by a selected month and year (RASFF Data)

The “Effects” button under the heading “Meteorological Parameters” shows the total effect/contribution of meteorological parameters obtained based on the importance scores (Section 4) on the total number of RASFF notifications on a monthly basis (for the main export season (October-January) (Figure 5.7 and Figure 5.8). The color code used in the graph (Figure 5.8) indicates the level of the contribution of the meteorological parameter as low (from pastel) to high (dark). Therefore, after clicking the button “Effects”, a bar graph opens including the total number of RASFF notifications by month (Figure 5.7). The user can access information on the contribution of the meteorological parameter by selecting a month in the main export period (October, November, December, and January) by clicking on it on the bar graph. Another graph (Figure 5.8) then opens that demonstrates the effect of the meteorological parameter obtained according to the importance score (Section 4) using the color code described above.

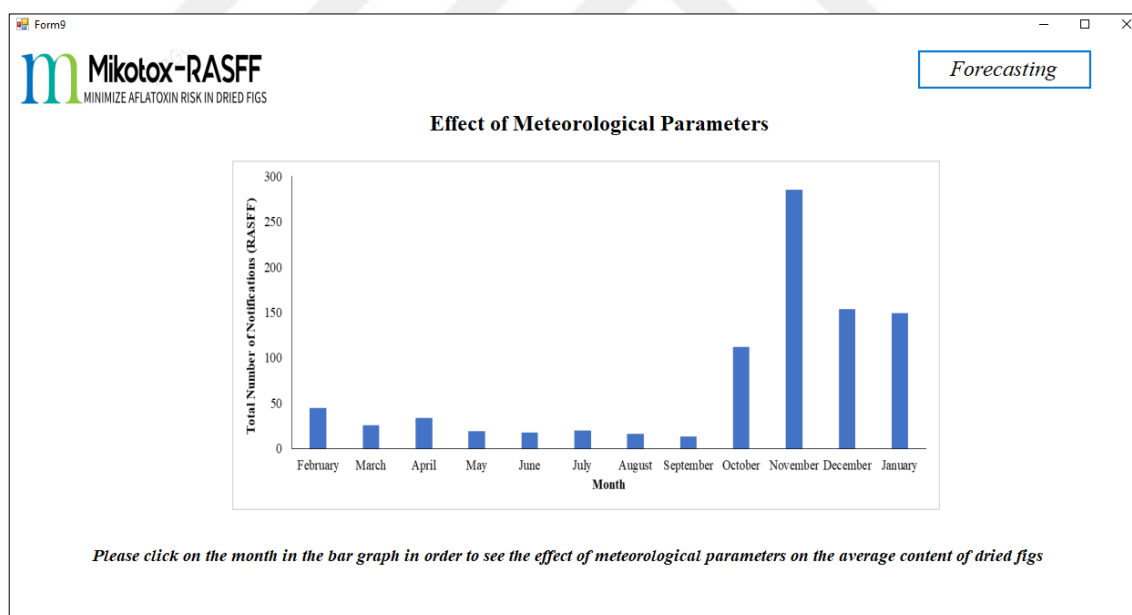


Figure 5. 7. The total number of RASFF notifications by months

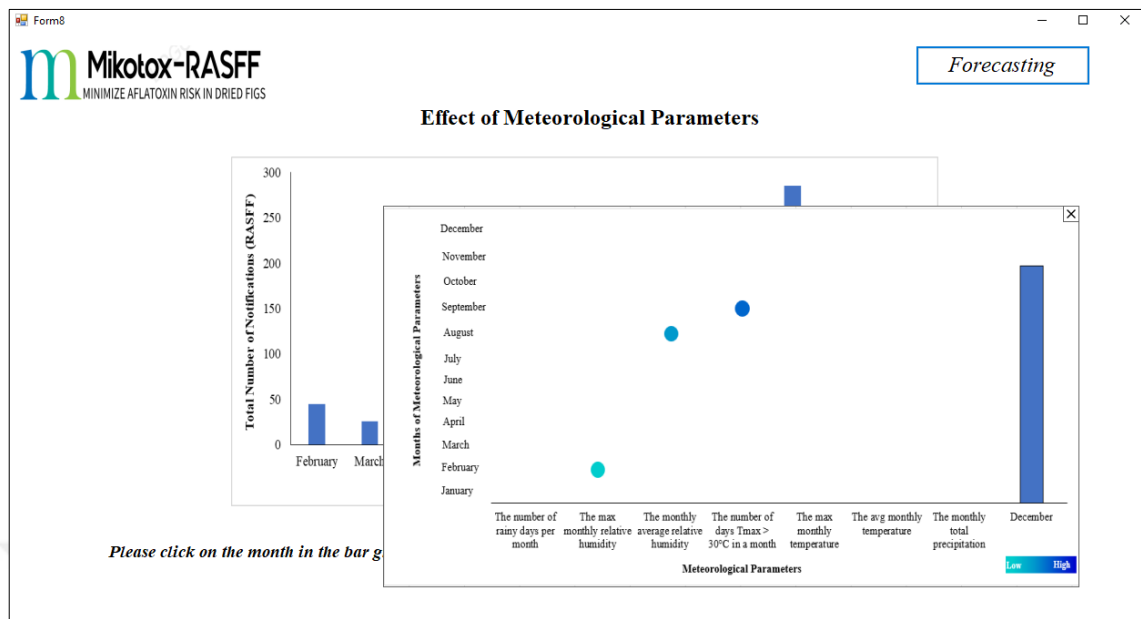


Figure 5. 8. Effect of meteorological parameters (from pastel (low) to dark (high)) on the total number of RASFF notifications

If the user clicks the “Forecasting” button, a page will open (Figure 5.9) including the forecasting bar graph (at the bottom right of the page) presenting the monthly total number of notifications for the next 12 months predicted using the developed RNN model (Section 4). The system generates alerts (using exclamation marks on the forecasting bar graph) when the change in the total number of notifications is statistically significant. The z-table will be used to calculate a statistically significant increase in the total number of notifications.

A line graph (top-left) showing the trends in the meteorological parameters for the previous year is linked to the forecasting graph to enable scenario-based forecasting. The effect of a change in meteorological parameters on the risk forecast can be observed on the forecast graph by changing the value of selected parameter(s) using the boxes provided next to each parameter. For example, if the user wants to see the effect of a 2°C increase in the average monthly temperature (eg. September, October, November, December, January), first he/she needs to select the parameter and the month(s) in the line graph then select the value that he/she wants to see the change of value in the box that is found near the related parameter. After the user changes the meteorological parameter, the effect of the change on the forecasting of the total number of notifications will be observed in the forecast graph with a degree of the change via different colors (eg. yellow) (Figure 5.10).

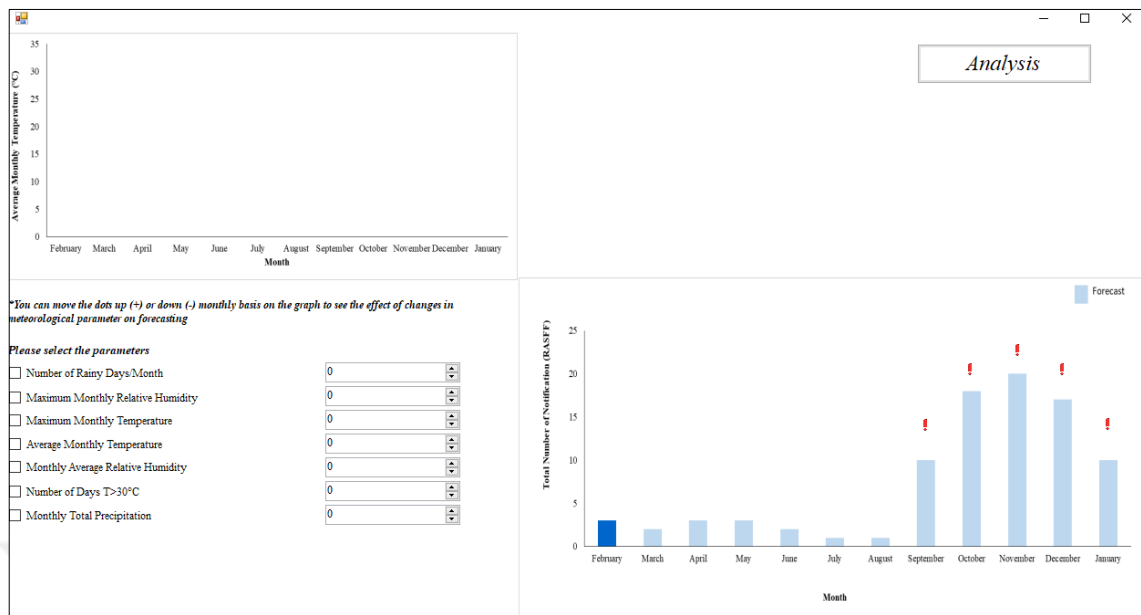


Figure 5. 9. Forecasting of RASFF notifications

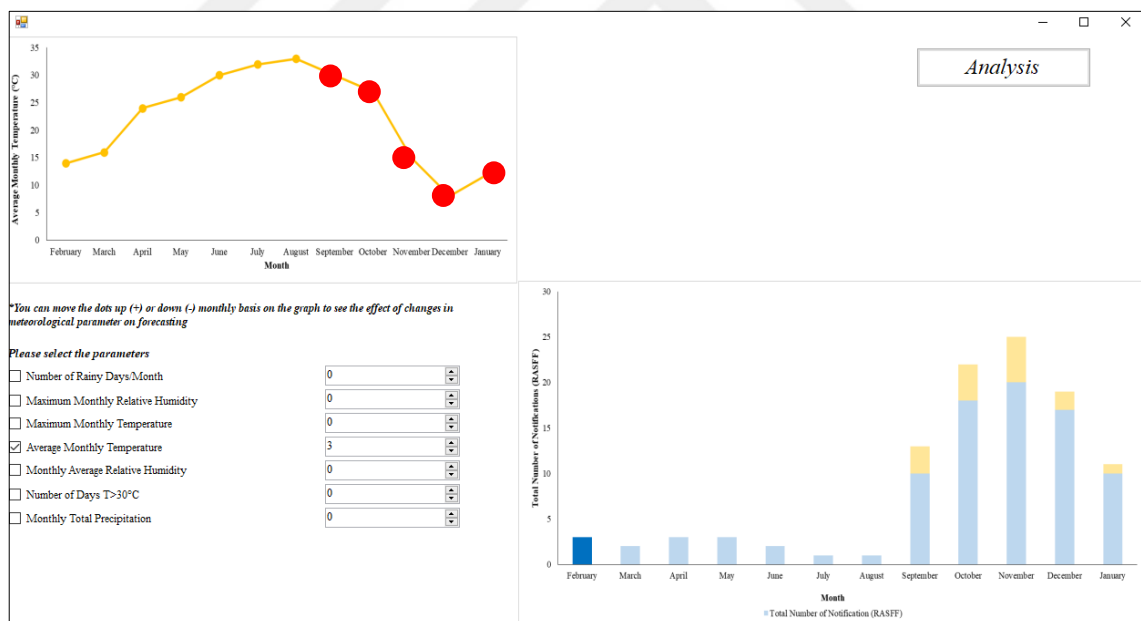
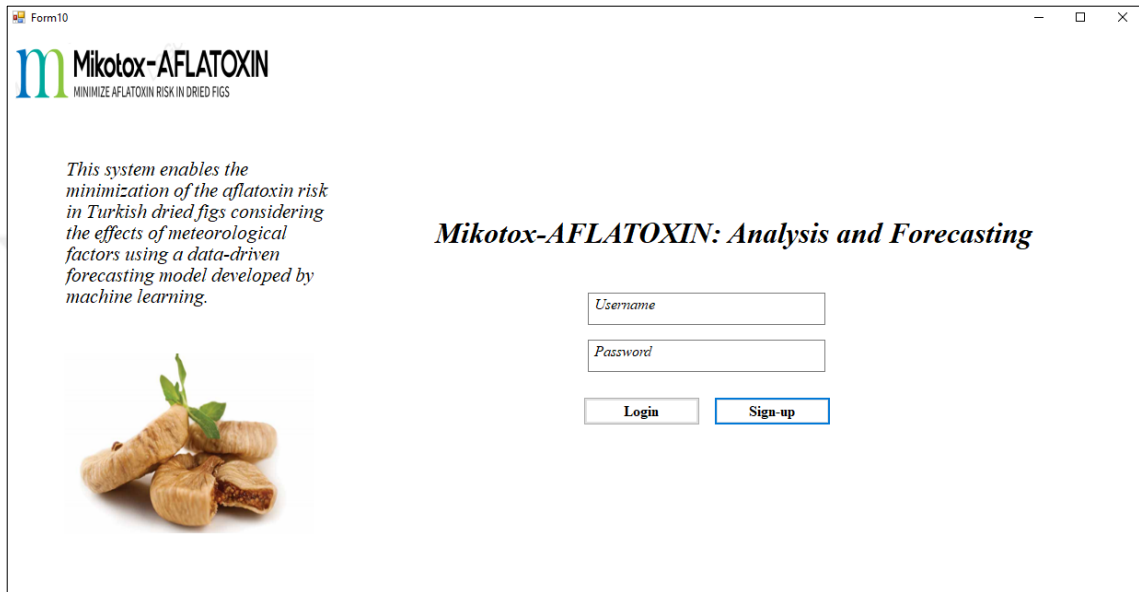


Figure 5. 10. Forecasting of RASFF notifications with different meteorological scenarios

#### 5.3.2.2. Module 2: Mikotox-AF- Monitoring and Forecast of AF Risk (Based on Total AF Contamination Data) in Dried Figs for Ministry Use

Similar to Module 1, the homepage of the Module 2 (Figure 5.11) includes a brief description of the system. The user starts using the system with the Register/Sign-up button. Users can

sign up using their email and then start using the system using the username and password they selected. Information on the user (including the sector they do belong to) is collected upon registration. Upon login, the module starts with a page that includes three buttons “Analysis”, “Forecasting”, and “Database for Other Factors” (Figure 5.12), which enables the user to select one of the three main functionalities of the system.



**Mikotox-AFLATOXIN**  
MINIMIZE AFLATOXIN RISK IN DRIED FIGS

*This system enables the minimization of the aflatoxin risk in Turkish dried figs considering the effects of meteorological factors using a data-driven forecasting model developed by machine learning.*

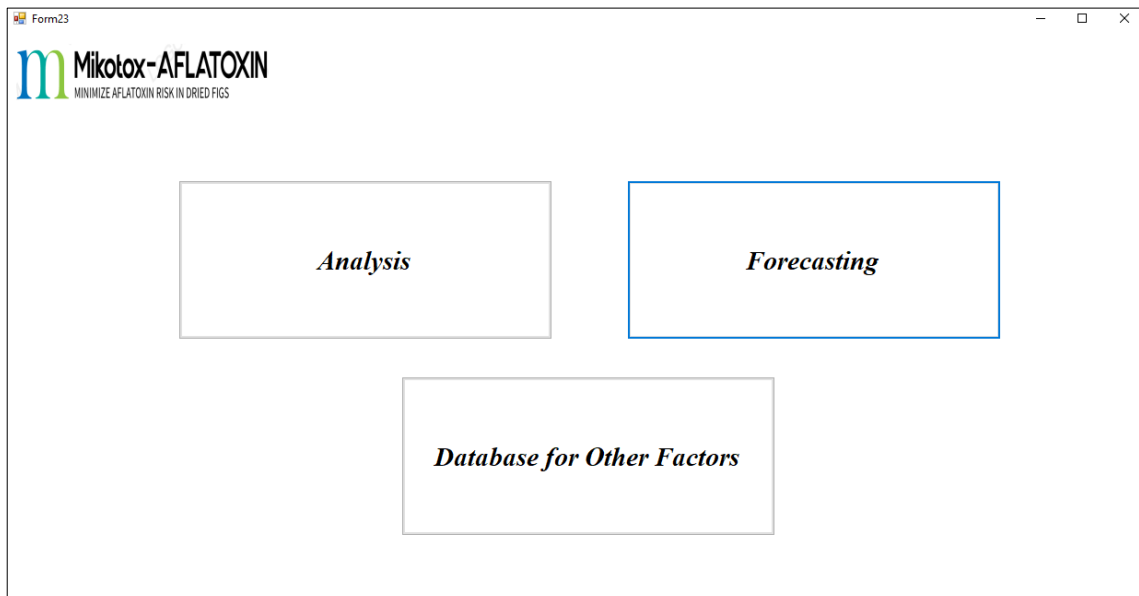
**Mikotox-AFLATOXIN: Analysis and Forecasting**

Username

Password

Login Sign-up

Figure 5. 11. Homepage of the Mikotox-AF System



**Mikotox-AFLATOXIN**  
MINIMIZE AFLATOXIN RISK IN DRIED FIGS

**Analysis**

**Forecasting**

**Database for Other Factors**

Figure 5. 12. The system main page including three buttons as “Analysis”, “Forecasting”, and “Database for Other Factors”

Similar to module 1 by clicking on the “Analysis” button and selecting the type of trend analysis (total AF yearly and monthly trends) user can access the analytical functionality (Figure 5.13). The user can also access the monthly and yearly trends of meteorological factors and analysis of the contribution of meteorological factors based on importance scores as described in Section 4 under “Meteorological Parameters”.

The yearly trends of the total AF concentration data were demonstrated using a bar graph showing the change in the total AF concentrations by year (Figure 5.14). The monthly trends of total AF concentration are presented by a bar graph including two sliders that user can select the month and year (Figure 5.15). This function will provide the comparison of total AF content based on the selected month and year to the user.

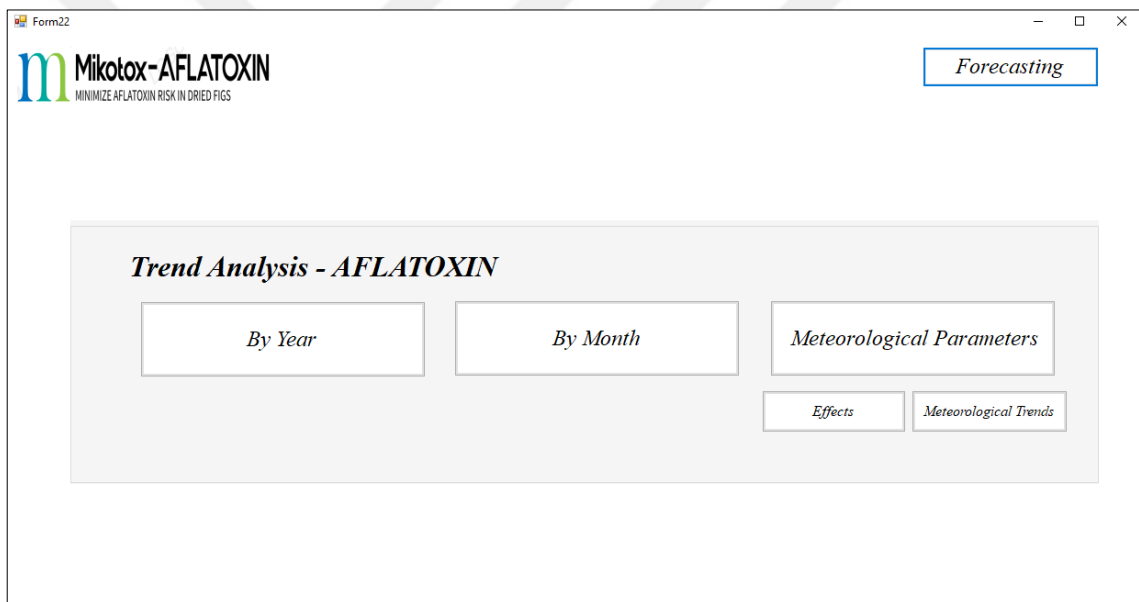


Figure 5. 13. Analysis of AF contamination data web page

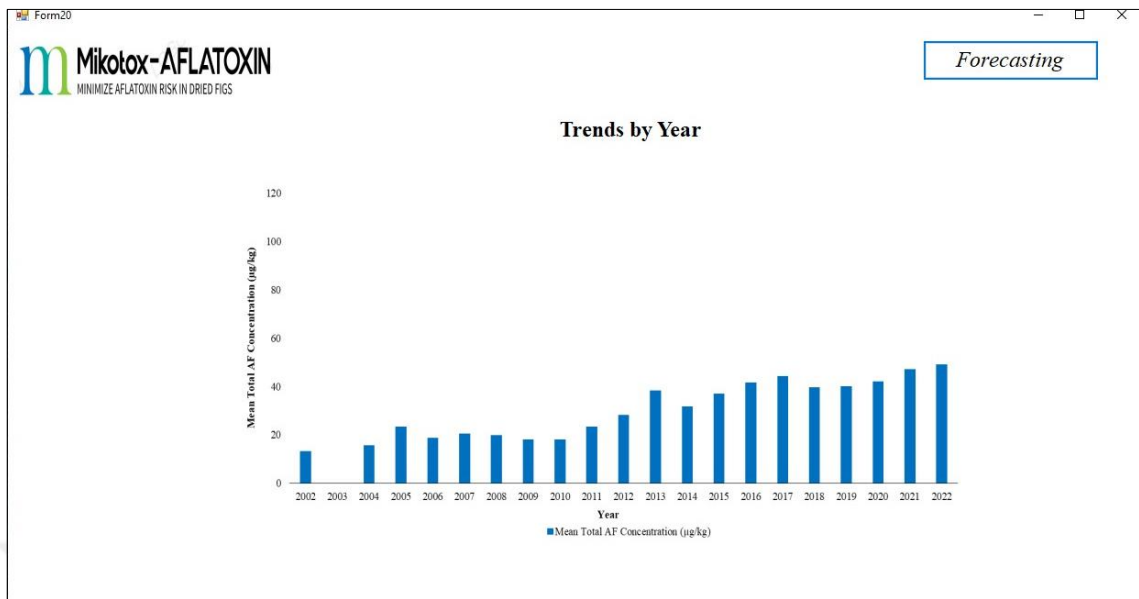


Figure 5. 14. Trends in the mean total AF content by year

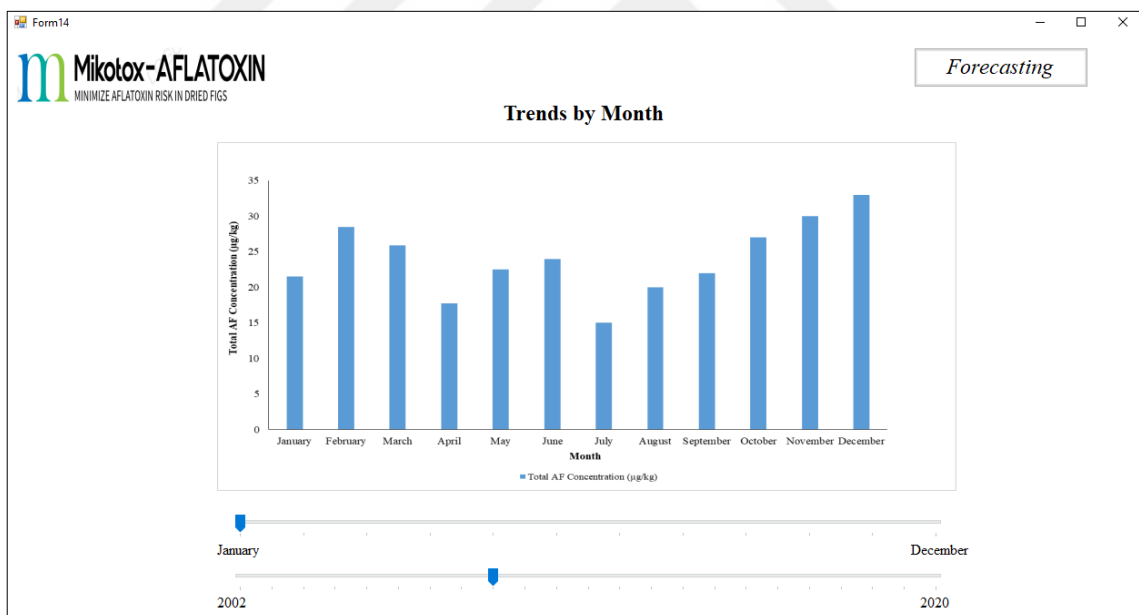


Figure 5. 15. Trends of total AF content by a selected month and year

Similar to Module 1, the trends of meteorological parameters are integrated as a bar graph demonstrating the yearly and monthly trends (line charts) (Figure 5.16) when the user clicks the “Meteorological Trends” button on the yearly and monthly trend analysis page (Figure 5.16). When the user selects one or more of the meteorological parameters by clicking in the

right part, he/she can see the trends in meteorological parameter(s) (monthly averaged) on the total AF concentration as a line graph considering the selected month and years (slider).

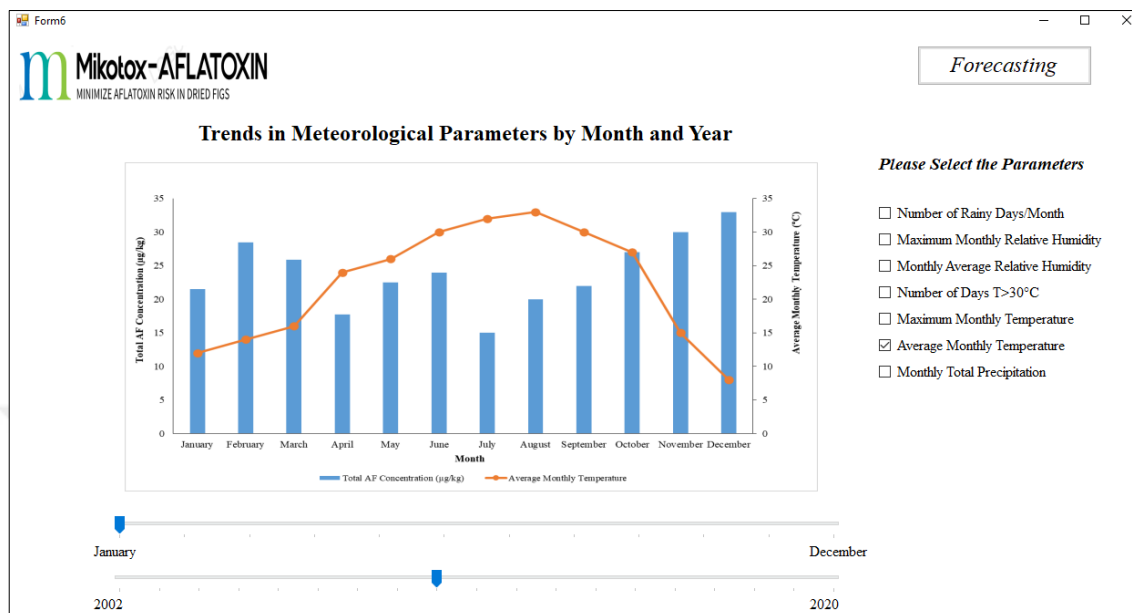


Figure 5. 16. Trends in meteorological data by a selected month and year (total AF)

Similar to module 1; the button for the “Effects” which is accessible under the “Meteorological Parameters” (Figure 5.13) shows the total effect/contribution of meteorological parameters on the total AF concentration on a monthly basis (for the main export season (October-January) according to the importance score of the parameters obtained from the developed model (Section 4) (Figure 5.17 and Figure 5.18). The color code used in the graph (Figure 5.18) shows the level of contribution of the meteorological parameters as low (from pastel) to high (dark). After clicking the “Effects” button, a bar graph including the total AF concentration by month opens (Figure 5.17). The user will access information about the contribution of the meteorological parameter by selecting a month among the main export period (October-November-December-January) by clicking in the bar graph. Figure 5.18 demonstrates the effect of meteorological parameters on the total AF concentration that will be obtained according to the importance score (Section 4) using the color code described above.

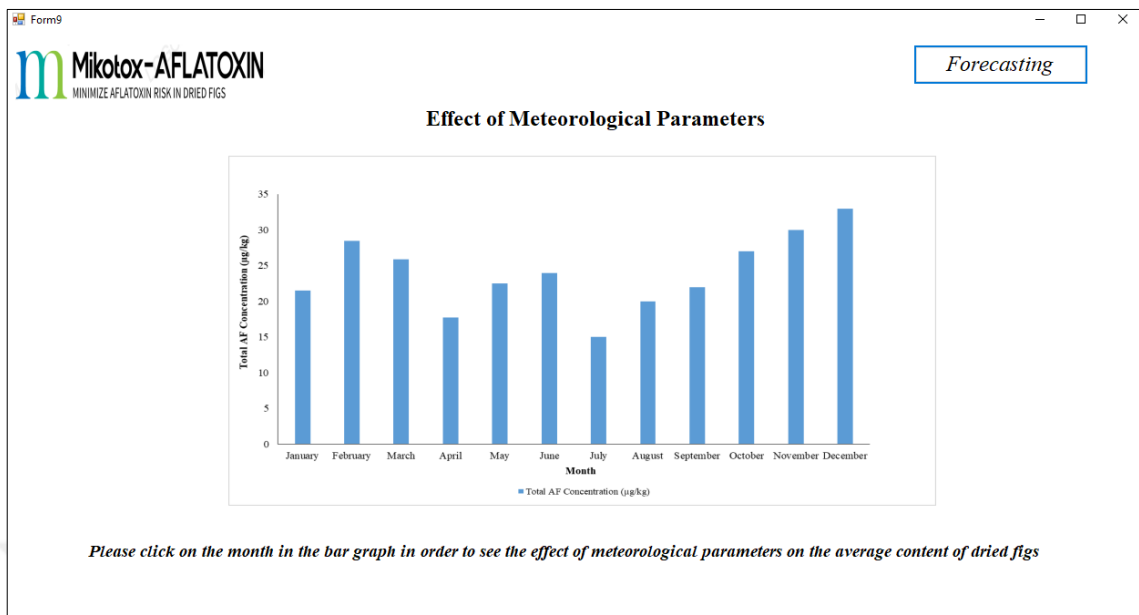


Figure 5. 17. The trends of mean total AF content by months

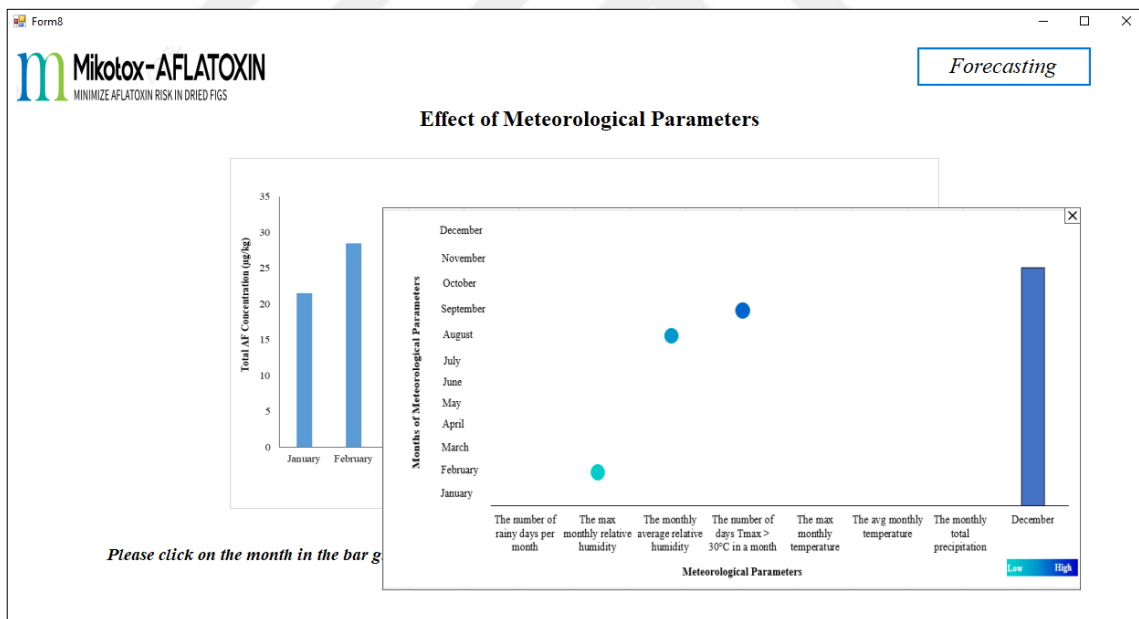


Figure 5. 18. Effect of meteorological parameters (from pastel (low) to dark (high)) on the prediction of total AF content

If the user clicks the “Forecasting” button, a page will open (Figure 5.19) including the forecasting bar graph (at the bottom right of the page) showing the monthly total AF content for the next 12 months predicted using the RNN model which was developed in Section 4.

Also, the system generates alerts by exclamation mark on the graph when the change in total AF concentration is statistically significant. To understand statistical significant increase in the total AF concentration, the z-table will be used.

A line graph presented in the top-left part of the page linked to the forecasting graph in order to monitor the scenario-based forecasting situations shows the trends in meteorological parameters for the previous year which. The effect of a change in any meteorological parameters on the risk forecast can be observed on the forecast graph by changing the value of selected meteorological parameter(s) using the boxes located in next to each meteorological parameter. For example, if the user wants to see the effect of a 2°C increase in the average monthly temperature (eg. September, October, November, December, January), similar to module 1, first he/she needs to select the parameter and the month(s) in the line graph then select the value that he/she wants to see the change of value in the box that is found near the related parameter. After the user changes the meteorological parameter, the effect of the change on the forecasting of the total number of notifications will be observed in the forecast graph with a degree of the change via different colors (eg. yellow) (Figure 5. 20).

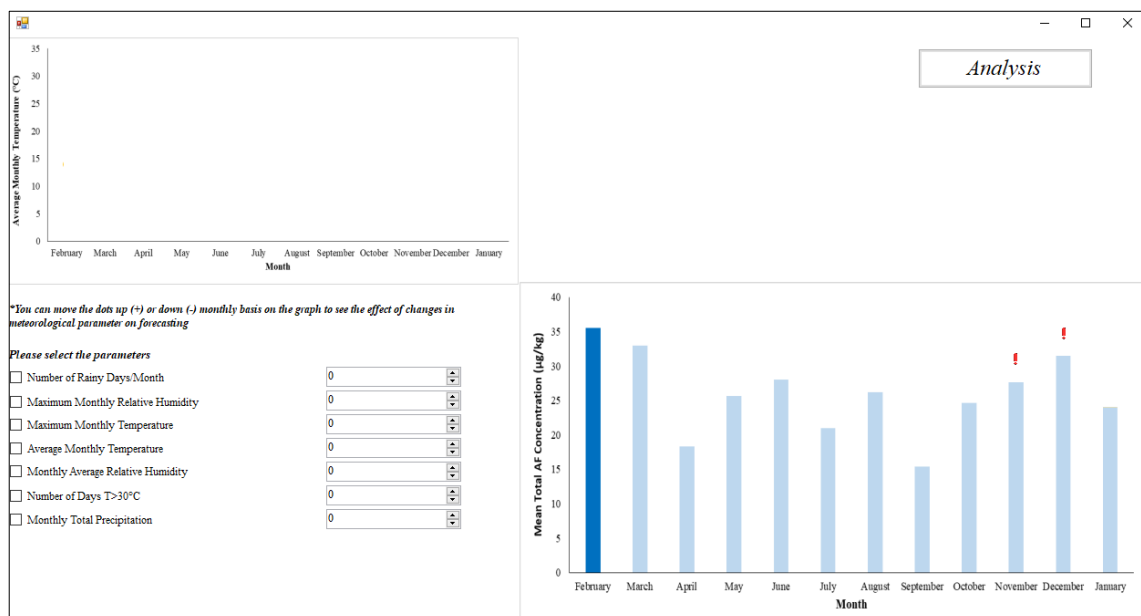


Figure 5. 19. Forecasting of AF contamination data

Different from Module 1; Module 2 includes a database to enable collection of data for factors (other than meteorological parameters) that could be included in the modeling to improve prediction accuracy and used for research purposes.

The user can enter yearly data (%) for the production and processing related factors identified based on earlier research (harvesting and drying method) by clicking on “Database for Other Factors” (Figure 5.21). Alternatively, the user can define the factor and data that will be entered into the database.

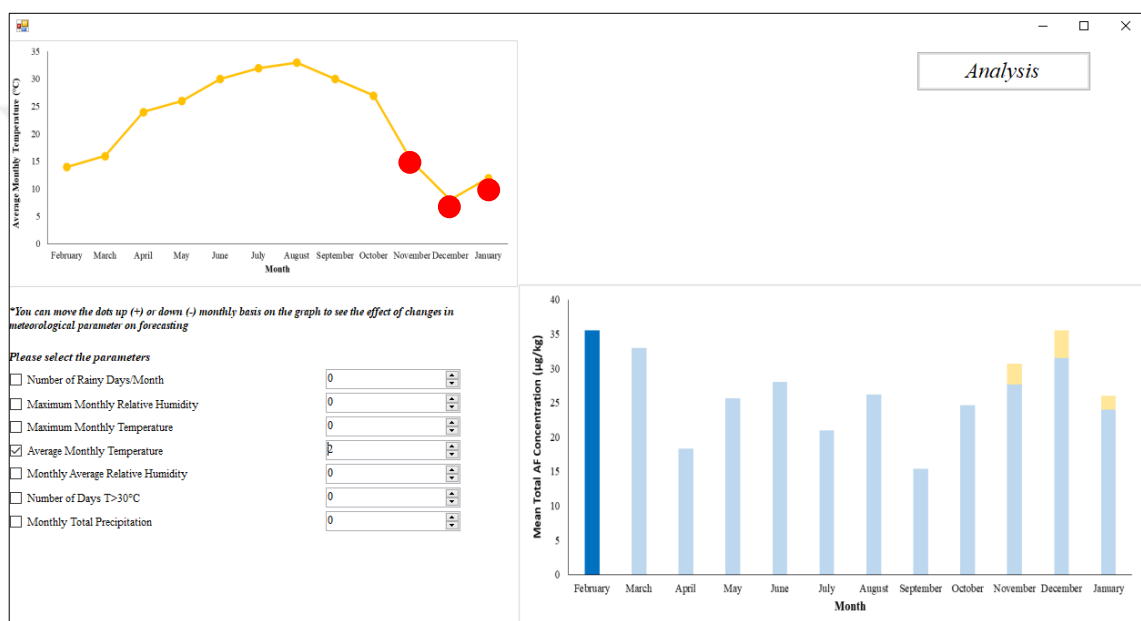


Figure 5. 20. Forecasting of AF contamination data

The screenshot shows a web form titled "Mikotox-AFLATOXIN" with the subtitle "MINIMIZE AFLATOXIN RISK IN DRIED FIGS". The form is titled "DATABASE FOR OTHER FACTORS" and contains three main sections:

- Harvesting method (%)**: Includes input fields for "Year", "Hand Harvesting (%)", and "Pick up From the Ground (%)".
- Drying Type (%)**: Includes input fields for "Year", "Sun drying and plastic crates (%)", "Sun drying on the ground (%)", and "Drying tunnel (%)".
- Enter Optional Data**: Contains three rows of input fields for "Year", "Process Category Name", and "Number of process" (with a dropdown menu showing "0").

Figure 5. 21. Database for other factors

## 5.4. CONCLUSION

In conclusion, the system comprises two distinct modules: Module 1 focuses on monitoring and forecasting RASFF-related risks for public use, while Module 2 is tailored for Ministry use, emphasizing monitoring and forecasting of AF risks based on Ministry contamination data. By analyzing contamination and meteorological trends and integrating warning signals, the system enhances forecasting capabilities, empowering ministry employees to guide industry actors using data from January onwards on a monthly basis. Meanwhile, stakeholders can utilize the RASFF analysis system (Module 1) for precautionary measures, monitoring forecasting trends monthly from January until Module 2's development is finalized. However, it's important to acknowledge system limitations. Future improvements should incorporate factors like soil-related parameters (e.g., soil temperature and humidity), as well as other agricultural parameters influential to AF contamination. While the current system targets dried figs, similar systems can be adapted for various food products, enhancing the mitigation of hazard risks and bolstering food safety measures.

## 6. GENERAL CONCLUSION

In this thesis, a predictive model and a web-based tool were developed to minimize the mycotoxin risk in dried figs with a systematic approach. An in-depth analysis of mycotoxin data specifically for Turkish dried figs was conducted using the open source RASFF database. Section 3 explored both product related (eg. weather conditions, agricultural and processing conditions) and non-product related factors (eg. export quantities, regulatory changes) influencing mycotoxin hazard based on yearly and monthly trends. Drought was the main weather-related factor that contributed to the high number of notifications in 2007 and 2012. However, this trend was not observed in the mean total AF concentration possibly due to data limitations. Factors that could influence mycotoxin contamination of Turkish dried figs in the investigated time frame were identified based on an in-depth review of academic and grey literature. While the effect of weather conditions on mycotoxin contamination of dried figs has been reported in the literature, a comprehensive study measuring the impact of different agricultural practices or weather conditions is lacking. To address this gap, collection and analysis of quantitative data on every factor influencing AF contamination starting from pre-harvest to transportation is recommended. Development of a comprehensive traceability system would facilitate data collection aiding both data recording and digital evaluation.

Considering the outputs of the first part of the thesis that suggested a correlation between the weather conditions and mycotoxin risk, a predictive model was developed using a machine learning approach (RNN) to understand the contribution of weather-related factors to mycotoxin risk in dried figs. This time series model, built using data from primary dried fig producing regions in Turkey, predicted the total number of RASFF notifications from monthly meteorological data with an r-value of 0.64. Temperature-related factors (such as the number of days when the maximum temperature higher than 30°C, average and maximum monthly temperature observed especially in drying season) especially came into prominence and contributed to the increase in the total number of notifications. While this study provides interesting insights into the contribution of individual weather parameters, statistical evaluation of the observed effects that considers the interaction between the parameters is required to obtain a full picture.

The use of RNN enabled the incorporation of time-dependencies and non-linearities in the dataset improving the predictive power of the model. Yet the performance of the model was limited by availability of mycotoxin hazard data. In this study, publicly available RASFF data on total number of mycotoxin hazard related notifications were used for modeling. The use of more extensive datasets, like control data from the Republic of Türkiye Ministry of Agriculture and Forestry database (i.e. mycotoxin concentration of control samples), is expected to improve the prediction performance considerably. Additionally, integrating uncertainty data of the measurement, if available, into the model could further improve predictive capability.

The model can be further improved by incorporating data on other factors that can influence mycotoxin contamination (e.g. soil-related parameters). The success of further modeling efforts would thus largely rely on systematic data collection and analysis for the agricultural and weather-related factors of interest. The forecast capability can be further improved by coupling the developed model with forecast models for the independent parameters (i.e. meteorological forecast models).

In the last part of the study, two modules were designed including forecasting of RASFF-related risks as Module 1 which can be used publicly, and forecasting of AF risks based on the contamination data for Ministry use as Module 2. In addition to the forecasting capability using the developed RNN model, both modules offer the opportunity to monitor monthly trends that the actors in the sector can use to minimize the mycotoxin risk in dried figs.

In conclusion, this tool has great potential as a comprehensive system to minimize mycotoxin risk in dried figs. It provides a powerful framework for the systematic study of mycotoxin contamination by enabling data collection and analysis. Addressing data gaps, particularly those highlighted in Sections 1-3, will provide deeper insights into contamination triggers and the overarching influence of various factors. Systematic collection and statistical analysis of data on mycotoxin contamination routes and influencing factors throughout, from pre-harvest to transportation, (eg. agricultural practices, processing methods (eg. the use of drying tunnels), and weather conditions) coupled with the forecasting capability would provide a strong foundation for control efforts aimed at minimizing mycotoxin risk. As more data is collected, the RNN model can be refined to bolster its predictive capacities. In the food sector, various safety concerns arise from diverse hazards.

The model and system proposed, while developed for dried figs, could be repurposed to tackle various food safety-related challenges.



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## 6. APPENDIX: PYTHON ALGORITHMS FOR MODELLING STUDIES

```

import numpy as np
import torch
import torch.nn as nn
from google.colab import drive
from scipy.stats import pearsonr
from sklearn.model_selection import TimeSeriesSplit

class Model:
    def __init__(self, k, m, p, x, y):
        self._device = torch.device("cuda" if
torch.cuda.is_available() else "cpu")
        self._network, self._predictions, self._weights = None, None,
None
        self.k, self.m, self.p, self.x, self.y = k, m, p, x, y

    def crossvalidate(self):
        self._network = Network(self.x.shape[1], self.p,
self.y.shape[1]).to(self._device)
        self._predictions = np.zeros_like(self.y)
        criterion = nn.MSELoss()
        optimiser = torch.optim.Adam(self._network.parameters())

        for training, test in TimeSeriesSplit(self.k).split(self.x):
            for _ in range(self.m):
                optimiser.zero_grad()
                criterion(torch.tensor(self.y[training],
device=self._device), self._network(torch.tensor(self.x[training],
device=self._device))).backward()
                optimiser.step()

            with torch.no_grad():
                self._predictions[test] =
self._network(torch.tensor(self.x[test],
device=self._device)).cpu().numpy()

    def explain(self):
        self._weights = np.zeros((self.x.shape[1], self.x.shape[0],
self.x.shape[0]), self.x.dtype)
        x = torch.tensor(self.x, device=self._device,
requires_grad=True)
        y = self._network(x)

```

```

        self._network.zero_grad()
        for i in range(x.shape[1]):
            for t_0 in range(x.shape[0] - 1):
                for t_n in range(t_0 + 1, x.shape[0]):
                    self._network.zero_grad()

                    if x.grad is not None:
                        x.grad.zero_()

                    y[t_n].backward(retain_graph=True)
                    self._weights[i, t_0, t_n] = x.grad[t_0,
i].cpu().numpy()

class Network(nn.Module):
    def __init__(self, n, p, q):
        super(Network, self).__init__()

        self._gru, self._linear = nn.GRU(n, p), nn.Linear(p, q)
        self.n, self.p, self.q = n, p, q

    def forward(self, x):
        return self._linear(self._gru(x)[0])

np.random.seed(42)
torch.manual_seed(42)

if torch.cuda.is_available():
    torch.cuda.manual_seed_all(42)

drive.mount("/content/drive")

k = 5
m = 1000
p = 100
x = np.load("x.npy")
y = np.load("y.npy")
model = Model(k, m, p, x, y)

model.crossvalidate()
model.explain()

r, p = pearsonr(model.y[32:].squeeze(),
model._predictions[32:].squeeze())
print(r, p)

```