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**Isolation and Identification of Mycotoxigenic Fungi Associated with
 Dried Tomato Chips from Gusau, Zamfara State, Nigeria**

Shinkafi, S. A.^{1*}, Bashir, Y. R.², Suleiman, N.², Salisu, N.¹, Sani, Z. R.², , Warra, A. A.², Aguh, I. B.² and Sufiyanu, S.^b

¹ Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria.

²Department of Biological Sciences, Federal University Gusau,
P.M.B 1001 Gusau, Zamfara State, Nigeria.

³ Center for Entrepreneurship Development, Federal University Gusau, Zamfara State, Nigeria.

Corresponding Authors Email Address: saadatushinkafi@rocketmail.com Mobile phone: +234806 945 1716

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ABSTRACT

Chipping and sun drying is the most popular and traditional method of tomato preservation in major tomato producing areas in northern Nigeria. The drying is done in open and on a mat or at times on bare floor including shoulder of roads or any surface that is unhygienic. Tomato is an important vegetable crop that is consumed worldwide. It has numerous vital vitamins and other nutrients that promote health and well being of humans. Tomato has been found to be beneficial in the management of diseases such as cancer, cardiovascular and so on. The improper drying of tomato and poor storage has in many reports been found to be more suitable for pathogenic fungi to grow and produce their secondary metabolites which may be poisonous to humans. A total of fifty (50) dried tomato samples were obtained from nine (9) different markets in Gusau, Zamfara state, Nigeria and screened for the presence of fungi. Isolation and identification was done using cultural and cellular examinations. Fungi Isolation was carried out on potato dextrose agar and malt extract agar, samples were inoculated and incubated for at 5 days at 25°C. Identification of the fungi was based on colonial morphology on the plates and microscopic observation in comparison with mycological key. Fungi isolated are mostly of *Aspergillus* and *Penicillium* genera. *Aspergillus niger* was found to be the highest occurring fungi with 52.3%, other fungi isolated were *Penicillium oxalicum*, *Aspergillus flavus* each accounting for 6.8% of isolated and identified fungi. *Penicillium chermesium*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Curvularia americana* with 4.5% each and *Rhodotorula glutinins*, *Fomitopsis meliae*, *Penicillium vericussum*, *Chaetominum*, *Aspergillus nidulans* and *Aspergillus sydowii* with 2.3% each. Some of these fungi species are known to produce toxins which could be harmful to both humans and livestock.

Key words: Tomato, Chips, Fungi, Disease, Toxins

1.0 INTRODUCTION

Tomato (*Solanum lycopersicum*) belongs to the family solanaceae. It is the second most important vegetable crop which provides 40 % of the daily value (Bhowmik, Kumar, Paswan, and Srivastava, 2012). Tomato is one of the most important and popular commercial vegetable grown in the world as well as in Africa (Hacksh *et al.*, 2015). It is rich in vitamin (A, B₁, B₂, and C), water, organic acids, minerals and dietary fiber FAOSTAT(2013). Tomato is used to make soup, puree, ketchup, juice and can be consumed either raw or cooked (Brckett, 2012). Consumption of tomato decreases the risk of breast, head, and neck prostate cancer. It also

helps in the formation of blood vessels, healthy immune system and sperm production (Drusch and Ragab, 2003). It also helps in decreasing conditions such as osteoporosis and cardiovascular diseases (Bhowmik *et al.*, 2012). Due to its high water content, which is estimated to be about 94% , it usually becomes susceptible to microbial contamination especially fungi and bacteria, viruses and nematodes, this also gives room for fungi to produce mycotoxins which are detrimental to human health (Onuorah, S. and Orji, M. 2005) To minimize these problems

tomatoes are processed into chips and sun dried and stored until off-season.

According to findings of (Hegazy, 2017), there is huge loss of fresh tomatoes as a result of fungal attack, this is due to its high moisture contents. Most occurring fungi in the contamination of tomatoes include *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Mucor* sp. and *Penicillium* sp. (Mbajiuka *et al.*, 2014; Onuorah and Orji, 2015; Saini, 2018)

Chipping and sun drying is the most popular and traditional method of tomato storage in major tomato producing areas in northern Nigeria. The drying is done in open and on a mat or at times on bear floor including shoulder of roads or any surface that is unhygienic. This method of drying exposes the chips to possible infestation by fungi and continue to contaminate them during storage. Some fungi produce antibiotics and mycotoxins. In recent years there have been increased concerns due to mycotoxin particularly because of their role in human and animal health, productivity, economics of their management and trade. Both humans and animals are constantly prone to invasion by microorganisms such as pathogenic fungi, bacteria, viruses and their secondary metabolites including mycotoxins (Pitt J.I., 2000). This research work aimed at isolating and identifying fungi found in dried tomato chips from Gusau, Zamfara State, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Gusau, the capital city of Zamfara state which is located on longitude 6°15' East and latitude 12° 10' North. It has an average annual temperature of 26.3 °C and can reach up to 40 °C in March. The average annual relative humidity is 15% and can reach up to 83% in August. The state has an average rainfall of 868 mm. The rainfall is started in April and ends in October (Mammanet *al.*, 2000). Based on the results of 2006 National Population Census Gusau Local Government had a population of about 383,162 people (NPC, 2006).

2.2 Sample Collection

A total of fifty (50) dried tomatoes samples were collected from nine (9) different markets in

Gusau, Zamfara state, Nigeria between the months of November, 2018 and January, 2019. The markets were coded for easier identification of samples. The number of samples collected from each market and the codes are; Tsohuwar Kasuwa (TK) 5 samples, Tudun Wada Market (TW) 6 samples, Central Market (CM) 6 samples, Kasuwar Danjumma (KD) 6 samples, Kasuwar Garejin Mailena (GRJ) 6 samples, YarHanya Market (YHM) 6 samples, Damba Market (DMB) 6 samples, YarDantsi Market (YRD) 6 samples and Janyau Market (JNY) 2 samples. The samples were collected in sterile polyethene bags and taken to Microbiology Laboratory, Department of Biological Sciences, Federal University Gusau for isolation (M. S *et al.*, 2017).

2.3 Materials Sterilization

Prior to the preparation, all glass wares used were properly washed, dried and sterilized in the oven at 160°C for one hour, surfaces of laboratory work bench were also disinfected with 70% ethanol to reduce contaminants (Dimphna, 2016).

2.4 Media Preparation

The media used for the research are potato dextrose agar (PDA) and malt extract agar (MEA). The media were prepared according to the manufacturer's instruction and incorporated with chloromphenicol. The media were aseptically dispensed onto sterile petridishes (Dimphna, 2016).

2.5 Sample Processing

One gram (1g) of each sample was cut using sterile scalpel and enriched for 24 hours in sterile potato dextrose broth as labeled appropriately. Thereafter, six (6) folds serial dilution of the samples were carried out (Onuorah, S. and Orji, M. 2005).

2.6 Isolation of Fungi

Pour plate method was adopted for the isolation of fungi as described by (Samuel and Orji, 2015). One milliliter of the serially diluted (10^4) was aseptically dispensed into a sterile bottle containing sterile media (PDA and MEA). The media was mixed thoroughly with the sample and aseptically transferred on to petridish, this was done for all the samples. The plates were

incubated in an inverted position at 25° for 5 days. Colonies were observed and sub-cultured repeatedly until pure cultures were obtained. The pure cultures were then sub-cultured on PDA slants and stored for identification and characterization (Onuorah, S. and Orji, M. 2005).

2.7 Identification of Fungal Isolates

Fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation (Tafinta *et al.*, 2013). The isolates were further identified by slide culture techniques as described by Oyeleke and Manga (2008). A portion of the aerial mycelium was picked using a sterile inoculating needle and inoculated on a slide containing a fraction of solidified potato dextrose agar. The slide was incubated for 48 hours at 25°. It was then viewed under light microscope using x10 objective lens and then x40 to detect hyphae, spores and other structures. The microscopy was done using lactophenol cotton blue stain. Morphological appearance of the fungal isolates from dried tomatoes were confirmed and

authenticated with the aid of Mycological Atlas of (Sarah *et al.*, 2016)

3.0 RESULTS

A total of 44 fungal isolates were recovered from the samples. *Aspergillus niger* is the highest occurring fungi with 52.3%, this is followed by *Aspergillus flavus* and *Penicillium oxalicum* (6.8%). *Penicillium chermesinum*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Rhodotorula glutinins*, *Curvularia Americana* have 4.5% and lastly *Fomitopsis meliae*, *Penicillium verrucosum*, *Chaetominum globosum*, *Aspergillus nidulans*, *Aspergillus sydowii* with 2.3% each (Table 1).

Figure 1 represents the prevalence of fungal isolates in tomato chips from nine markets in Gusau. It shows that samples collected from Kasuwar Danjumma (KD) have the highest prevalence of fungi with 22.7%, this is followed by samples from Damba (DMB) and Tsohuwar Kasuwa (TK) with 15.9%. Janyau market (JNY) was found to have the least prevalence 2.3%. Table 2 shows the distribution of fungal isolates from different locations.

Table 1: Frequency of Fungi Isolated from Fried Tomato Chips

Organisms	Number of occurrence	Frequency (%)
<i>Penicillium chermesinum</i>	2	4.5
<i>Aspergillus parasiticus</i>	2	4.5
<i>Aspergillus niger</i>	23	52.3
<i>Aspergillus oxalicum</i>	3	6.8
<i>Aspergillus fumigatus</i>	2	4.5
<i>Rhodotorula glutinins</i>	2	4.5
<i>Aspergillus flavus</i>	3	6.8
<i>Curvularia Americana</i>	2	4.5
<i>Fomitopsis meliae</i>	1	2.3
<i>Penicillium verricosum</i>	1	2.3
<i>Chaetomium globosum</i>	1	2.3
<i>Aspergillus nidulans</i>	1	2.3
<i>Aspergillus ydowii</i>	1	2.3
Total	44	100

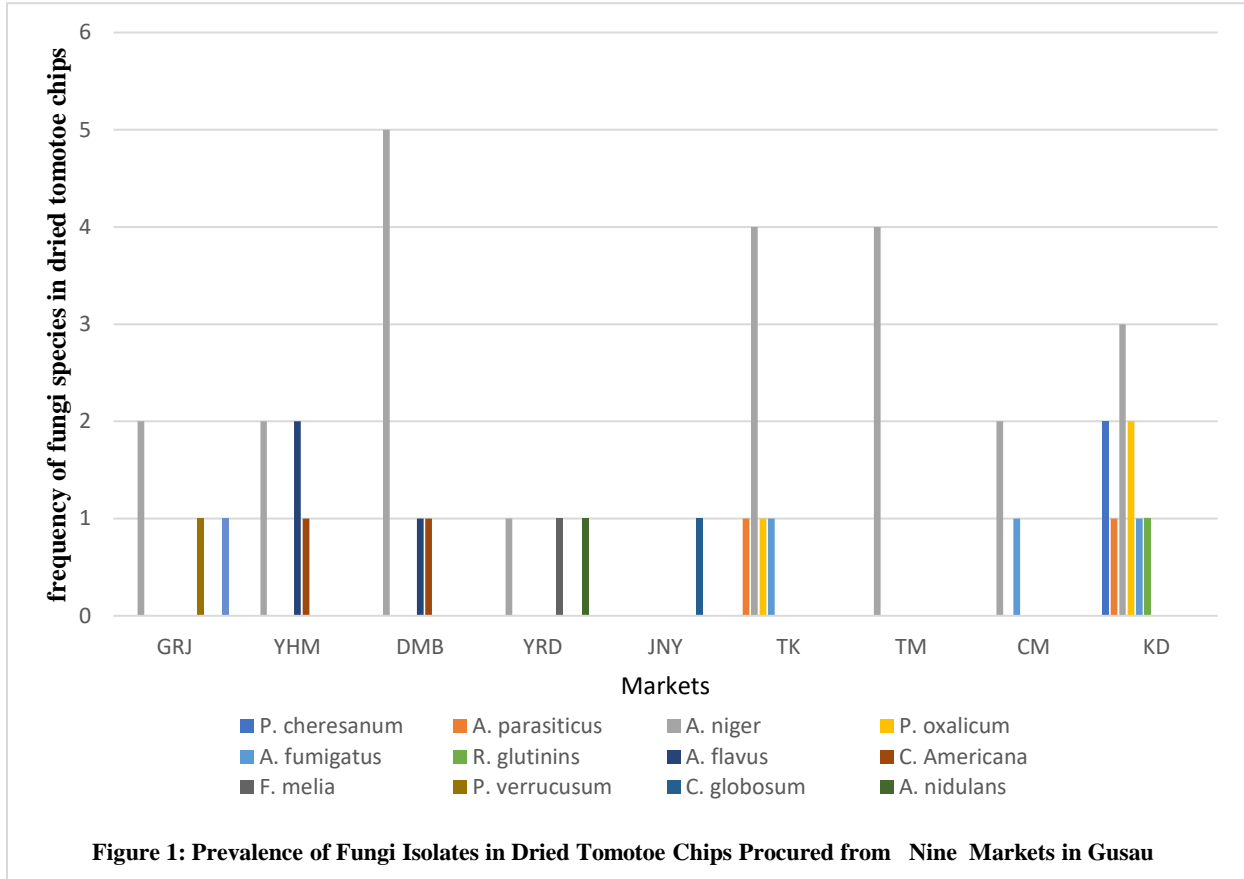


Figure 1: Prevalence of Fungi Isolates in Dried Tomotoe Chips Procured from Nine Markets in Gusau

KEY:

GRJ: GAREJIN MAILENA MARKET, **YHM:** YAR HANYA MARKET, **DMB:** DAMBA MARKET, **YRD:** YAR DANTSI MARKET, **JNY:** JANYAU MARKET, **TK:** TSOHUWAR KASUWA, **TM:** TUDUN WADA MARKET, **CM:** CENTRAL MARKET, **KD:** KASUWAR DANJUMMA

4.0 DISCUSSION

The study was conducted using cultural cellular methods for the identification of fungi isolated from dried tomato chips from nine markets in Gusau, Zamfara state, Nigeria. Thirteen species belonging to six genera were isolated from the samples. In this work, six species from the genera *Aspergillus* were isolated and identified; these include *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans* and *Aspergillus sydowii*. However, three species were also isolated and identified from the genera *Penicillium*; these are *Penicillium oxalicum*, *Penicillium vericuccum* and *Penicillium chermesinum*. Lastly, one isolate each was identified from the following; *Rhodotorula glutinins*, *Curvularia americana*, *Fomitopsis meliae* and *Chaetominum globosum*. In a similar study carried on isolation and identification of fungi associated with postharvest decay of tomatoes fruit sold in Abakaliki Nigeria, *Aspergillus*, *penicillium* and *fusarium* were more dominant among the isolated fungi. These organisms and others identified are mycotoxigenic fungi which if present in abundance, could lead to Mycotoxins production which may produce a wide range of harmful effects in both humans and animals among which are immune-toxicity and Nneka, (2016). Our result is also in conformity with previous reports by (Mailafia, S. *et al.*, 2017), (Sanyaolu, 2016) who stated that most isolates recovered from dried vegetables including tomato are

Aspergillus spp. It also agrees with the finding of (Chigoziri, John, and Nam, 2018). On the occurrence of fungi, *Aspergillus niger* has the highest frequency with 52.3%. This agrees with the research conducted by (Mailafia, Okoh, Olabode, and Osanupin, 2017), (Onuorah, S. and Orji, M. 2005). It also goes in contrary with that of (Oyemaechi, Chukwuezi, and Ozougwu, 2014) discovered the absence of microorganisms in fresh healthy tomatoes, but in spoiled tomatoes, they discovered 6 species of fungi. Most fungi isolated are pathogenic to tomato; the pathogenicity could be from the condition of storage and drying of tomato. Several reports have indicated that most drying process of tomato are done on either road sides or bare soil (Etebu, Nwauzoma, and Bawo, 2013).

5.0 CONCLUSION

The practice of drying tomato on bare floor or road side gives room for soil pathogenic fungi to contaminate the fruit and cause infection if consumed. The present work identified *Aspergillus spp.* and *Penicillium spp.* as the most occurring species of fungi found in dried tomato chips sold in 9 different markets effects.

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