Isolation of Mycotoxigenic Fungi, Detection and Quantification of Ochratoxin A from Coffee (Coffea arabica L.)

Legese Hagos1, *, Nagassa Dechassa2, Kifle Belachew1, Demelash Teferi1, Solomon Yilma2, Gabisa Gidisa1

1Ethiopian Institute of Agricultural Research, Jimma Agricultural Research Center, Jimma, Ethiopia
2Ethiopian Institute of Agricultural Research, Ambo Plant Protection Research Center, Ambo, Ethiopia

Email address:
legesehagos2006@yahoo.com (L. Hagos)
*Corresponding author

To cite this article:
Legese Hagos, Nagassa Dechassa, Kifle Belachew, Demelash Teferi, Solomon Yilma, Gabisa Gidisa. Isolation of Mycotoxigenic Fungi, Detection and Quantification of Ochratoxin A from Coffee (Coffea arabica L.). Journal of Drug Design and Medicinal Chemistry. Vol. 6, No. 4, 2020, pp. 47-52. doi: 10.11648/j.jddmc.20200604.12

Received: December 30, 2020; Accepted: January 12, 2021; Published: January 22, 2021

Abstract: Coffee is the most important commodity and source of export earnings in Ethiopian economy which has to fulfills the quality standards of safety up to maximum tolerable level. However, it is naturally associated with several mycoflora and some of them may produce Ochratoxin A unless careful handling measures taken place. Therefore, this research was initiated to assess the status of mycotoxigenic fungi associated with coffee and quantification of Ochratoxin A from locally consumed coffee in Ethiopia. A total of 75 coffee samples were collected from three districts namely, Haru, Homa and Nedjo of West Wollega Zone, Oromia regional state of Ethiopia. Determination of coffee associated mycoflora isolation and identification were conducted at Jimma Agricultural Research Center of plant pathology laboratory while Ochratoxin A detection and quantification were conducted at Ambo Plant Protection Research Center. Malt Extract Agar (MEA) was used for isolation and identification of mycoflora associated with coffee and ELISA kit was used to detect and quantify Ochratoxin A. The result showed that numbers of mycoflora associated with coffee were observed and five of them become the major. Aspergillus niger was the most dominant (73.37%) species detected from most coffee samples, followed by Aspergillus ochraceus (11.30%), Fusarium spp. (7.37%), Penicillium spp. (6.74%), and Rhizopus spp. (1.50%), respectively. Average ochratoxinA recorded was 0 (ND) ppb, 1.24 ppb and 2.02 ppb from Haru, Homa and Nedjo.

Keywords: Coffee, Ethiopia, Mycotoxigenic Fungi, Ochratoxin A

1. Introduction

Globally, coffee is the second most traded and wealth generating commodity after oil [1, 2]. Ethiopia is known for its diverse and unique Arabica coffee flavors [3]. Coffee covers 37% of agricultural exported commodities [4]. Ethiopia is the largest coffee producer in Africa and the 4th in the world next to Brazil, Colombia and Honduras by contributing 7.4% of total world coffee production [5]. Sixteen percent of the Ethiopian population depend on coffee Production, Processing and Marketing and Ethiopia exported around 180,000 metric tons of coffee and earns 800 million US$. [6].

Several biotic and abiotic factors affect coffee production and quality in the field and after harvest. Fungal contamination and production of mycotoxins is one of the post-harvest problems that influence the quality of coffee beans. Most of the storage fungi, mainly several species of Aspergillus and Penicillium do not invade seeds to any appreciable degree before harvest. However, they can cause severe discoloration of seed in storage resulting in germination failure, due to damaged embryos or whole seeds [7]. Temperature, moisture content, storage conditions, Processing type, and durations of storage can be
important factors for mold development. Ochratoxin A frequently occurs as a contaminant in various food products and it is the main mycotoxin reported to be found in coffee in green bean, roasted coffee and instant coffee worldwide [8-10].

Ochratoxin A has carcinogenic, nephrotoxic, teratogenic, immunotoxic, and neurotoxic properties. It has been associated with nephropathy in humans. OTA is a secondary metabolites of certain fungi which is a small molecule soluble in water and it is chemically constituted by a combination of an amino acid (phenylalanine) and a polyketide to carbon 10, contains one chlorine atom necessary for its biological activity, and it is stable when exposed to heat [11]. The presence of OTA in coffee has a negative economic effect for the producing countries and consumers. Many food processors in Europe and around the world are regularly testing for the presence of OTA in commodities like coffee before destined for human consumption [12]. International Agency for Research on Cancer [13] classified OTA as group 2B (possible carcinogens to human).

Due to the economic impact, OTA takes global attention which is associated with public health. The world community is becoming sensitive for mycotoxin contamination. Many food processors in Europe and around the world are regularly testing for the presence of ochratoxin A in commodities like coffee before destined for human consumption [12]. EU has set maximum permitted level of OTA 5µg/kg for roasted and ground coffee and 10 µg/kg for soluble coffee [14]. The current study was initiated with the objective of isolation and identification of Arabica coffee bean associated fungi and detection and quantification of Ochratoxin A from locally consumed green coffee beans.

\[
\text{Percent of Fungal infection(\%) = } \frac{\text{number of beans from which fungus isolated}}{\text{Total number of beans analyzed}} \times 100
\]

The growing fungi were sub cultured on Malt Extract Agar for 7 days and identified to species level based on cultural and morphological characteristics, including colony color, conidiophores, phialides, presence and size of vesicles, surface texture, color and the reverse side of mould on agar plate, mycelium, type of spore, pigmentation, Vesicle, Hyphae, Metulaes, Arrangement of conidia [15-18]. The pure cultures of each isolate were preserved at 4°C on MEA slant during the study.

2.4. Detection and Quantification of Ochratoxin A by ELISA kits

ELISA kits were used as a quick diagnostic method and most commonly used for detection of mycotoxins. The full kits containing Ochratoxin Low Matrix Microplate - 9610CH01COFN: 96 wells specific antibody, positive and negative control were used for detection of ochratoxin A. Samples extraction and processing were done as per the company procedure or order. The detection reagent is a covalent complex of these Ochratoxin A and enzyme, alkaline phosphates. The reagent was mixed with a sample extract and the mixture was placed in the well. In the control well in mycotoxin free sample, the mycotoxins-enzyme conjugate has been saturated the bound antibody, after additions of a chromogenic substrate results in the development of color and has been quantified by the use of ELISA reader.

3. Results

3.1. Coffee Bean Associated Fungi

The result of this study revealed that total of four fungal genera were recorded from Haru, Homa and Nedjo districts of West Wollega. According to the study, the percentages of fungal genera observed 84.67%, 7.37%, 6.74% and 1.5% of Aspergillus spp, Fusarium spp, Penicillium spp, and Rhizopus spp, respectively (Figure 1). Accordingly, Aspergillus spp. were the most dominant fungal genera and relatively Rhizopus was the least. The fungal genera of Aspergillus found to be the most frequently isolated from...


coffee bean followed by Fusarium and Penicillium, respectively where as Rhizopus was relatively the least one.

Figure 1. Percentage of total coffee bean associated fungal genera.

3.2. Mycotoxigenic Fungi Associated Coffee Bean

Aspergillus ochraceus and Aspergillus niger were found to be coffee bean associated mycotoxigenic fungi. Aspergillus niger was 73.3% while Aspergillus Ochraceus was 11.37%.

3.3. Coffee Bean Associated Fungi from Different Districts of West Wollega

Based on district level the composition of mycotoxigenic fungi observed were A. ochraceus (3.9%, 18.1%, 5.7%), A. niger (64.1%, 72.3%, 91.0%), Penicillium (13.8%, 2.8%, 3.0%), Fusarium (16.8%, 4.1%, 0.4%) and Rhizopus (1.4%, 2.6% 0.0%) from Haru, Homa and Nedjo, respectively (figure 2). Aspergillus niger was the most dominant in all districts while Rhizopus was the least one. Aspergillus ochraceus was also found in all districts with highest percentage in Homa, district followed by Nedjo and Haru.

Figure 2. Percentage of coffee bean associated fungi from different district.
3.4. Correlation Matrix

3.4.1. Relationship Between Mycotoxigenic Fungi and Ochratoxin A

The result of this study revealed that there was highly significant difference positive weak correlation between Aspergillus ochraceus on one hand and ochratoxin A ($r=0.31$) on the other (Table 1).

### Table 1. Ochratoxin A concentration using ELISA kit.

|          | A.ochraceus | A.niger | Ochratoxin A | Moisture content | Visible mold overgrowth |
|----------|-------------|---------|--------------|------------------|------------------------|
| A.ochraceus | 0.14        | 0.31**  | 0.25*        | 0.11             |                        |
| A.niger   | 0.14        | 0.12    | 0.10         | 0.20             | 0.44***                |
| Ochratoxin A | 0.31**      | 0.23*   | 0.27*        | 0.44***          |                        |
| Moisture content | 0.25*       | 0.20    |              |                  |                        |
| Mold condition | 0.11        | 0.27*   |              |                  |                        |

Where: A.niger=Aspergillus niger, A.ochraceus=Aspergillus ochraceus. ** Significant at P<0.01, * Significant at P<0.05.

3.4.2. Relationship Between Mycotoxigenic Fungi and Mold Condition

The result of this study revealed that there was significant difference positive weak correlation between Aspergillus niger on one hand and ochratoxin A ($r=0.27$) on the other (Table 1).

3.4.3. Relationship Between Mycotoxigenic Fungi and Moisture Content

There was also significant difference positive weak correlation between Aspergillus ochraceus on one hand and moisture content ($r=0.25$) on the other.

3.4.4. Relationship Between Ochratoxin A and Mold Condition

There was positive weak correlation between ochratoxin A on one hand and mold condition ($r=0.20$) on the other.

3.4.5. Relationship Between Ochratoxin A and Moisture Content

Similarly, there was significant difference positive weak correlation between ochratoxin A on one hand and moisture content ($r=0.23$) on the other.

3.4.6. Relationship Between Mold Condition and Moisture Content

There was highly significant difference positive intermediate correlation between moisture content on one hand and mold condition ($r=0.44$) on the other (Table 1).

3.5. Level of Ochratoxin A from Green Coffee

The result revealed that the level of Ochratoxin A were different from district to district. Haru district was found to not detectable amount while Homa was found 1.23 µg/kg (1.23 ppb) OTA level and Nedjo was relatively the highest one which was detected a mean of 2.03 µg/kg (2.03 ppb). The occurrence of Aspergillus ochraceus is not necessarily mean that there is ochratoxin A but the factors like moisture content may play a role for the production of Ochratoxin A. Thus while Aspergillus ochraceus observed from the samples collected from Haru, but we couldn’t detect any ochratoxin A from the district.

### Table 2. Ochratoxin A concentration using ELISA kit.

| Districts | No of sample analyzed | Average OTA concentration in µg/kg (ppb) |
|-----------|-----------------------|----------------------------------------|
| Haru      | 25                    | 0 (ND) Not detected                    |
| Homa      | 25                    | 1.23 µg/kg (1.23 ppb)                  |
| Nedjo     | 25                    | 2.03 µg/kg (2.03 ppb)                  |
| Mean      |                        | 1.08 µg/kg (1.08 ppb)                  |

*ppb=part per billion

4. Discussion

Four major fungal genera Aspergillus, Fusarium, Penicillium and Rhizopus were detected in association with coffee beans studied in West Wollega Zone. Aspergillus spp was dominantly associating with coffee beans which accounts (84.67%), Fusarium (7.37%), Penicillium (6.74%) and Rhizopus (1.50%). The results were in line with Geremew et al.[19] who reported that Aspergillus (79%), Fusarium (8%), Penicillium (5%) found in association with coffee beans. Urbano et al.[20] also reported that isolated filamentous fungi in coffee beans from Brazil they found that A. ochraceus and A. niger.

The moisture content of the coffee bean plays great role for the production of OTA as well as for the growth of mold formation over the cherry of coffee. Aspergillus niger was also significantly correlated with mold formation of the coffee cherries. This indicates that, coffee without mold formation may develop Ochratoxin A and moisture content is the most factors for OTA production. OTA has been detected and quantified by ELISA. The present study indicated that the concentration of OTA from green coffee that ND (not detected), 1.23 µg/kg and 2.03µg/kg from Haru, Homa and Nedjo, respectively. This result is in agreement with Geremew et al.[19] who reported concentration of OTA in locally consumed coffee from Ethiopia was 1.5µg/kg.

A significant feature of ochratoxin is that it occurs in a
wide variety of commodities such as raisins, barley, soy products and coffee in varying amounts but at relatively low levels [21]. Culliao and Barcelo [22] reported that OTA was more commonly detected in Robusta coffee (37%) than in Arabica coffee (26%). The highest level of OTA found in the dried whole cherries of Arabica 97 µg/kg, while for Robusta the highest level of 120 µg/kgOTA was found. Romani, et al. [23] also reported that the detected OTA from green coffee beans was 18–48 µg/kg in Congo. The current study revealed that when toxigenic strains of Aspergillus ochraceus showed positive significant correlation with OTA. This shows that the presence of toxigenic strains involves a great risk of OTA presence when it get favorable conditions. These mycotoxigenic fungi have a potential to produce high amount of OTA.

5. Conclusion

In conclusion, 75 coffee cherry samples has been collected from three districts of West wollega from, Haru, Homa, and Nedjo. The samples has been tested for Arabica coffee been associated mycoflora and level of concentration of ochratoxin A. There was a significant difference among the treatments on Aspergillus ochraceus, A. niger, Penicillium spp., Fusarium sp. and Rhizopus sp. from Haru. There was also a significant difference on A. ochraceus, A. niger, Penicillium sp., and Rhizopus spp. from Homa and there was a significant difference on A. ochraceus, A. niger, Penicillium spp., Fusarium sp. from Homa. While there was no significant difference among treatments on Fusarium spp. from Homa and Rhizopus spp. from Nedjo. The result showed that numbers of mycoflora associated with coffee were observed while five of them become the major. Among these Aspergillus niger were the most dominant (73.37%) detected from most coffee samples, while Aspergillus ochraceus (11.30%), Fusarium spp. (7.37%), Penicillium spp. (6.74%), and Rhizopus spp. (1.50%) were identified. ELISA kit has been used to detect and quantify ochratoxin A. The average ochratoxin A concentration detected was 0 (ND) Not detected 1.23 µg/kg (1.23 ppb) and 2.03 µg/kg (2.03 ppb) from Haru, Homa and Nedjo respectively.

Acknowledgements

The authors are thankful to Ethiopian institute of agricultural research, Jimma agricultural research center for financial support.

References

[1] Davis, A. P., Gole, T. W., Baena, S. and Moat, J., 2012. The impact of climate change on indigenous arabica coffee (Coffee arabaica): predicting future trends and identifying priorities. PLoS one, 7 (11).
https://journals.plos.org/plosone/article/file?type=printable&id =10.1371/journal.pone.0047981.

[2] Esquivel, P. and Jiménez, V. M., 2012. Functional properties of coffee and coffee by-products. Food Research
International, 46 (2), pp. 488-495.
https://www.sciencedirect.com/science/article/abs/pii/S09639 96911003449.

[3] International Coffee Genomics Network (ICGN). (2017). Report 10thCoffee Genomics Workshop held at the XXV Plant and Animal Genome (PAG) Meeting San Diego, California. January 14-18.

[4] USDA, N. (2012). The plants database. National plant data team, Greensboro, NC 27401-4901 USA.

[5] Foreign Agricultural Service (FAS). (2019). Coffee Annual Report. GAIN Report: ET1904.

[6] ICO (International Coffee Organization). 2016. Historical Data on the Global Coffee Trade. Available on http://www.ico.org/new storical.asp (2016). Accessed date on January, 2020. http://www.ico.org/new_historical.asp.

[7] Malaker, P. K., Mian, I. H., Bhuiyan, K. A., Akanda, A. M. and Reza, M. M. A. (2008). Effect of storage containers and time on seed quality of wheat. Bangladesh Journal of Agricultural Research, 33: 469-477.
https://www.banglajol.info/index.php/BJAR/article/view/1606.

[8] Taniwaki, M. H., Pitt, J. I., Teixeira, A. A. and Iamanaka, B. T. (2003). The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. Int J Food Microbiol. 82: 173-179.
https://www.sciencedirect.com/science/article/abs/pii/S01686 0502003100.

[9] Almeida, A. P.; Alaburda, J.; Shundo, L.; Ruvieri, V.; Navas, S. A.; Lamardo, L. C. A. M. (2007). Ochratoxin A in Brazilian instant coffee. Braz. Microb, 3: 300-303. https://doi.org/10.1590/S1517-8382200700200022.

[10] Amézqueta, S., González-Peña, E., Murillo-Arbizu, M. and de Cerain, A. L. (2009). Ochratoxin Adecontamination: A review. Foodcontrol, 20: 326-333.
https://www.sciencedirect.com/science/article/abs/pii/S09567 1508001631.

[11] Bueno, D., Muñoz, R. and Marty, J. L., 2014. Common methods to detect mycotoxins: a review with particular emphasis on electrochemical detection. Sensing in Electroanalysis. (K. Kalcher, R. Metelka, I. Švancara, K. Vytras; Eds.). 2013/2014, Volume 8.
https://dk.upce.cz/handle/10195/58390.

[12] Joint FAO/WHO Expert Committee on Food Additives. Meeting, 2001. Safety evaluation of certain mycotoxins in food (No. 74). Food & Agriculture Org. Control, 69: 65- 73.

[13] IARC, International Agency for Research on Cancer. (1993). Some naturally occurring substance: Food items and constituents, heterocyclic aromatic amines, and Mycotoxins: IARC Monographs on the Evaluation of carcinogenic Risk of chemicals to Humans. pp. 571. https://www.cabdirect.org/cabdirect/abstract/19952006807.

[14] EC. (2006). Maximum limits for ochratoxin A. Regulation EC official Journal of European Union, 25: 3 https://eur- lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364: 0005:0024:EN:PDF.

[15] Raper, K. B. and Fennell, D. J. (1965). The genus Aspergillus. The genus Aspergillus.
[16] Klich, M. A. (2002). Identification of Common *Aspergillus* species. Netherlands Centraal bureau voor Schimmel culture.

[17] McClenny, N. (2005). Laboratory detection and identification of *Aspergillus* species by microscopic observation and culture: the traditional approach. *Medical mycology, 43* (sup 1), pp. 125-128. https://doi.org/10.1080/13693780500052222.

[18] Pandit, P., Panta, O. P. and Karki, T. B., (2014). Isolation of *Aspergillus ochraceus* and Production of Ochratoxin in Coffee Samples. *Nepal Journal of Science and Technology, 15*: 133-138. https://www.nepjol.info/index.php/NJST/article/view/12030.

[19] Geremew, T., Abate, D., Landschoot, S., Haesaert, G., & Audenaert, K. (2016). Occurrence of toxigenic fungi and ochratoxinA in Ethiopian coffee for local consumption. *Food Control, 69*: 65-73. https://www.sciencedirect.com/science/article/abs/pii/S0956713516301943.

[20] Urbano, G. R., Taniwaki, M. H., Leitao, M. F. D. F. & Vicentini, M. C. (2001). Occurrence of ochratoxin A producing fungi in raw Brazilian coffee. *Journal of Food Protection, 68*, 1226–1230. https://meridian.allenpress.com/jfp/article/64/8/1226/170043/Occurrence-of-Ochratoxin-A-Producing-Fungi-in-Raw.

[21] CAST (Council for Agricultural Science Technology) (2003) Mycotoxins: Risks in plant, animal, human systems. Task Force Report, Ames, Iowa, No. 139, 1-199.

[22] Culliao, A. G. L.; Barcelo, J. M (2015). Fungal and mycotoxin contamination of coffee beans in Benguet province, Philippines. *Food Addit. Contam. Part A 32*, 250–260. https://www.tandfonline.com/doi/abs/10.1080/19440049.2014.1001796.

[23] Romani, S.; Sacchetti, G.; López, C. C.; Pinnavaia, G. G.; Dalla Rosa, M (2000). Screening on the occurrence of ochratoxinA in green coffee beans of different origins and types. *J. Agric. Food Chem. 48*: 3616–3619. https://pubs.acs.org/doi/abs/10.1021/jf990783b.