

Analysis of Coffee Drinking Habits, Age and Village Location on Proteinuria in coffee farmers in the District of BPR Ranau Tengah Kab. OKUS

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ABSTRAK

Kopi merupakan salah satu minuman yang sangat populer di dunia dengan produksi kopi yang mengalami kenaikan di tiap tahunnya. Proses pasca panen kopi sangat rentan terhadap serangan jamur Okratoksin A (OTA) yang dapat menyebabkan gangguan kesehatan. Tujuan penelitian ini adalah untuk menganalisis kebiasaan minum kopi, umur dan lokasi desa terhadap kandungan proteinuria pada petani kopi di Kecamatan BPR Ranau Tengah Kab.OKUS. Jenis penelitian ini adalah *cross sectional* dengan sampel sebanyak 304 responden diambil dengan metode *proportional stratified random sampling*. Pengambilan data menggunakan kuesioner dan observasi serta pengambilan sampel bubuk kopi dan sampel urin petani. Data dianalisis menggunakan uji *Chi-Square* dan sampel bubuk kopi diperiksa di Laboratorium MIPA UNSRI sedangkan sampel urin petani diperiksa di Laboratorium RS Dr. Maulana AK Baturaja. Hasil penelitian menunjukkan ada hubungan yang signifikan antara kebiasaan minum kopi dan umur dengan kandungan proteinuria dengan *p value* 0,000 sedangkan lokasi desa tidak ada hubungan yang signifikan dengan kandungan proteinuria *p value* 0,179. Hasil pemeriksaan bubuk kopi petani menunjukkan indikasi tercemar OTA dengan adanya pertumbuhan jamur *Aspergillus section nigri* dan *Aspergillus ochraceus* sebanyak 107 (35,2%).

Kata kunci : kopi, proteinuria, okratoksin A

ABSTRACT

Coffee is one of the most popular drinks in the world with coffee production increasing every year. The post-harvest process of coffee is very susceptible to attack by the fungus Okratoxin A (OTA) which can cause health problems. The purpose of this study was to analyze coffee drinking habits, age and village location on proteinuria content in coffee farmers in BPR Ranau Tengah District OKUS Regency. This type of research is cross sectional with a sample of 304 respondents taken by the proportional stratified random sampling method. Collecting data using questionnaires and observations as well as taking samples of coffee grounds and urine samples of farmers. The data were analyzed using the Chi-Square test and samples of coffee grounds were examined at the MIPA UNSRI Laboratory while the urine samples of farmers were examined at the Laboratory of Doctor Maulana AK Baturaja Hospital. The results showed that there was a significant association between coffee drinking habits and age with proteinuria content with *p value* of 0.000, while there was no significant association between location and proteinuria with *p value* of 0.179. The results of the examination of coffee grounds showed indications of contamination with OTA with the growth of the fungus *Aspergillus section nigri* and *Aspergillus ochraceus* as much as 107 (35.2%).

Keywords : *coffee, proteinuria, okratoxin A*

1. INTRODUCTION

Indonesia is the fourth largest coffee producer in the world with the habit of drinking coffee in its society that has been carried out for generations¹. The post-harvest process of coffee is very susceptible to microbial infection. Among these microorganisms, *Aspergillus* species such as *Aspergillus carbonarius*, *A. niger*, *A. ochraceus* and *A. westerdijkiae* are fungal species that produce ochratoxin A in tropical

and semi-tropical coffee plantations². Research conducted showed the presence of

fungal contamination of *A. niger* and *A. ochraceus* on coffee beans consumed by farmers in the District of BPR Ranau Tengah Kab. OKUS³. The effect of consumption of food products contaminated with ochratoxin A can cause health problems in humans and animals with various forms of clinical and pathological changes, for example it can cause liver cancer, liver degeneration, fever, swelling of the brain, kidneys, and nervous disorders where the kidney is the target organ. from OTA⁴. OTA exposure in humans can come from a variety of foods, such as; corn, wheat, rice, bread, mango, tomatoes,

grapes, watermelon, nuts, sesame seeds, coffee beans, cocoa, peanuts, chickpeas, soybeans, milk, smoked or salted fish and raisins. Even research stated that the presence of OTA was also detected in bottled water⁶.

The presence of OTA has been widely reported at various stages of coffee processing which can occur before or after the coffee harvest process, this depends on the environment or storage conditions, especially if the coffee beans are not dried according to standards (SNI: 01-2907-2008). Factors such as fungal species and strains, water activity, environmental factors (such as climatic conditions, storage and transportation) and processing (wet and dry processing) are factors associated with the presence of OTA contamination in coffee^{7,3}. One of the Robusta coffee-producing areas in South Sumatra is OKU Selatan Regency with the quality of the coffee produced being in grades 4, 5 and 6 (category quality currently until low). The low quality of coffee, among others, is caused by the low quality of plant material and poor post-harvest handling so that the coffee produced is susceptible to OTA contamination. The coffee bean storage process that is commonly carried out by farmers in the BPR Ranau Tengah sub-district is to simultaneously store raw coffee beans, roasted coffee beans and coffee grounds in one room. It is strongly suspected that this storage condition caused the recontamination of OTA-producing fungi into coffee grounds stated that toxic fungi can contaminate before the coffee brewing process, which is caused by several factors including the lack of heating during roasting and contamination after roasting; such as packaging, transportation and storage processes with unhygienic environmental conditions^{4,8,9,10}. This is what causes the importance of research on coffee drinking habits on proteinuria content. Proteinuria is the presence of protein in the urine in abnormal amounts with a value of more than normal levels, which is more than 150 mg/24 hours¹¹.

Data on proteinuria at the BPR Ranau Tengah Health Center is almost non-existent, this is because patients who come usually have advanced stages so they are immediately referred to hospitals outside OKUS Regency.

The purpose of this study was to analyze coffee drinking habits, age and village location on proteinuria content in coffee farmers in BPR Ranau Tengah District, OKUS Regency.

2. METHODOLOGY

Study Design and Sample

This type of research is *cross sectional* with independent variables including coffee drinking habits, age and village location, while the dependent variable is the proteinuria content of coffee farmers in Kec. Central Ranau BPR. The population who became respondents in this study were members of the coffee farmer group in the district. BPR Ranau Tengah as many as 1270 coffee farmers, so the Slovin formula was used for sampling.

$$n = N / (1 + (N \times e^2))$$

n = minimum number of samples,

N = total population

e = *error margin* (5%)

$$\begin{aligned} n &= 1270 / (1 + (1270 \times 0.05^2)) \\ &= 1270 / (1 + (1270 \times 0.0025)) \\ &= 304 \text{ samples} \end{aligned}$$

A sample of 304 respondents was taken using the *proportional stratified random sampling method* which is a sampling method by taking into account the region (village) and in determining the sample, representatives from each region are taken randomly. The samples taken are respondents with criteria of age ≥ 40 years with the habit of drinking coffee that comes from the processing by the farmers themselves

Urine Sampling

The urine sample taken is Urine Time, namely urine that is issued at a time that is not specifically determined¹². The collected urine is then put into a urine collection bottle

with a wide mouth and can be tightly closed, watertight and disposable with a urine collection volume of 50 ml. Then an examination is carried out using a dipstick test using *Reagent Strips* which is inserted

into the urine collection bottle. After that, coding was carried out which included the name, age of the respondent, village location and time of collection¹³.

3. RESULTS

The results showed that the population in Buay Pematang Ribu Ranau Tengah District was 22,055 people, with a composition of 11,518 people who were

male and 10,537 people who were female. Thus the population of Buay Pematang Ribu Ranau Tengah District is more male than female (Table 1)

Table 1 : Population characteristics according to Gender and Age Group in Buay Pematang Ribu Ranau Tengah District, 2019

Age	Male	Female	Amount
0 – 4	984	983	1967
5 – 9	1 015	963	1 978
10 – 14	999	932	1 931
15 – 19	938	849	1 787
20 – 24	1 008	827	1 835
25 – 29	980	795	1 775
30 – 34	1 016	810	1 826
35 – 39	831	794	1 625
40 – 44	797	725	1 522
45 -49	727	697	1 424
50 -54	624	642	1 266
55 – 59	569	539	1 108
60 – 64	483	395	878
65 – 69	256	263	519
70 – 74	153	160	313
75+	138	163	301
Amount	11 518	10 537	22 055

Source : Badan Pusat Statistik Kab. OKU Selatan

The educational characteristic of the majority of the population is that it only reaches the junior high school level, while the majority of the population's work is farming, being farmers of coffee, vegetables and other foodstuffs.

3.1. The Association of Coffee Drinking Habits on Protein Content in Urine in Coffee Farmers in Kab. OKUS

Visual observation showed the presence of foamy urine and the presence of protein content in the urine through urinalysis. In Table 1, the results of the *Chi-Square*

analysis show that the proportion of respondents who drink coffee with the presence of protein in the urine is 97 (31,9%) respondents, higher than those who do not drink coffee with the presence of protein in the urine, namely 10 (3,3%) respondents. This statistical test shows that there is a significant Association between coffee drinking habits and protein content in the urine of farmers in the District of BPR Ranau Tengah Kab. OKUS. So that farmers with the habit of drinking coffee have a 12,5

times greater probability of having protein in their urine than farmers who do

not drink coffee.

Table 1. The Association of Coffee Drinking Habits with Protein Content in Farmers' Urine in BPR Ranau Tengah District

Variable	Proteinuria n(%)	Unproteinuria n(%)	p value	OR
Drinking coffee	97 (31,9)	86 (28,3)	0,000	12,520 (6,160-25,446)
Don't Drink Coffee	10 (3,3)	111 (36,5)		
Amount	107(35,2)	197(64,8)		

Chi square test , = 0,05

3.2. The Association between Age and Protein Content in the Urine of Coffee Farmers in BPR Ranau Tengah District

Respondents involved in this study were only 40 years of age and over, from the mean value it was known that the age at risk for protein content in urine was ≥ 52 years. Based on the results of *Chi-Square analysis* (Table 2) it is known that the proportion of respondents who have a risk age of ≥ 52 years with the presence of protein in the urine is 96 (31,6%) respondents, higher than

those who have a less risky age < 52 years with the presence of protein content in the urine is 11 (3,6%) respondents. This statistical test shows that there is a significant Association between age and protein content in the urine of coffee farmers in the District of BPR Ranau Tengah Kab. OKUS. So that farmers with coffee drinking habits aged ≥ 52 years have 21 times the possibility of containing protein in urine compared to those aged < 52 years.

Table 2. The Association between Age and Protein Content in the Urine of Coffee Farmers in BPR Ranau Tengah District

Variable	Proteinuria n (%)	Unproteinuria n(%)	p value	OR
Age at Risk ≥ 52 Years	96 (31,6)	57 (18,8)	0,000	21,435 (10,690- 42,983)
Less Risk Age < 52 Years	11 (3,6)	140 (46,1)		
Amount	107 (35,2)	197 (64,8)		

Chi square test = 0,05

3.3. The Association between Village Location and protein content in urine on coffee farmers in BPR Ranau Tengah District

The location of the respondent villages that contained protein in urine in the District of BPR Ranau Tengah, namely Simpang Sender Tengah, Jepara, Tanjung Setia, Serumpun Jaya and Simpang Sender and Sukarame villages with the number of sufferers ≥ 10 respondents. Based on the

results of *Chi-Square analysis* (Table 3) it is known that the proportion of respondents living in risky locations with protein content in urine is 64 (21,1%) respondents, not different from those living in less risky locations with the presence of protein in the urine. urine is 45 (14,1%) respondents. This statistical test shows that there is no significant Association between the location of the village and the presence of protein

content in the urine of coffee farmers in the District of BPR Ranau Tengah Kab. OKUS.

Table 3. Association between Village Location and protein content in urine on coffee farmers in BPR Ranau Tengah District

Variable	Proteinuria n(%)	Unproteinuria n (%)	p value
Village Locations at Risk	64 (21,1)	102 (33,6)	0,179
Less Risky Village Locations	45 (14,1)	95 (31,2)	
Amount	107 (35,2)	197 (64,8)	

Chi square test = 0.05

4. DISCUSSION

4.1. The Association of Coffee Drinking Habits on Protein Content in Urine in Coffee Farmers in Kab. OKUS

The habit of drinking coffee on the average respondent is ≥ 4 cups per day which has been introduced since they were toddlers, with the reason to avoid seizures during fever. The coffee that is usually drunk is black coffee that comes from processing coffee beans owned by the farmers themselves, and it is suspected that there is ochratoxin A, which OTA is accumulative in the body¹⁴. The existence of OTA is strongly suspected to have come from the storage process in the same warehouse space between raw materials for dry coffee beans, roasted coffee and ground coffee so that recontamination occurs (Figure 1).

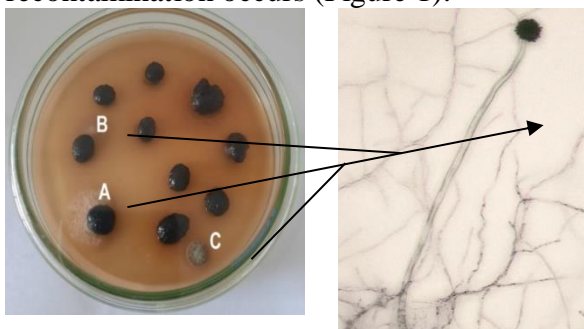


Figure 1: A,B,C = roasted coffee beans that have been stored; 1 = *Aspergillus section nigri* (Source: Personal document)

Figure 1. shows the presence of fungi that contaminate roasted coffee beans that have been stored for 1 week. The storage of

these coffee beans together with raw coffee beans, roasted coffee beans and coffee grounds are stored together in one room. Toxic fungi can contaminate before the coffee brewing process, which is caused by several factors including the lack of heating during roasting and contamination after roasting; such as packaging, transportation and storage processes with unhygienic environmental conditions^{8,9,10}.

Exposure to ochratoxin A can occur through food and drink because the toxin from the *A. niger species* is cosmopolitan in which fungal spores can be spread by the wind¹⁵. while *A. ochraceus* is a type of fungus whose habitat is in the soil¹⁶. Foods and beverages that are susceptible to OTA include grapes, corn, wheat, nuts, poultry, pork, dairy products, coffee, beer and spices (Fuchs and Peraica, 2006). Even research stated that the presence of OTA was also detected in bottled water⁶.

The results of research showed the presence of mycotoxins with the respective percentages of aflatoxin, ochratoxin and fumonisin in 31 patients' urine were 61.29%, 93.5% and 19.4%¹⁸. Likewise research with respondents with impaired kidney function showed an increase in OTA levels of 18 ± 7 ppb in serum with levels higher than the normal value of 3.3 ± 1.5 ppb (the average value of OTA) for healthy control respondents¹⁹.

The target organ of OTA (ochratoxin A) is the kidney with the main nephrotoxic effect in the post proximal nephron and

proximal tubule²⁰. In addition, in the circulatory system, OTA is almost completely bound to albumin. The level of albumin binding determines the plasma half-life of OTA, which in humans has a longer half-life than in other species. Albumin is the most abundant plasma protein in the circulatory system. Albumin can bind OTAs with very high affinity, therefore as much as 99.8% of OTAs are in bound form with albumin in the human circulatory system²¹.

At various stages of coffee processing, the presence of OTA can occur before or after the coffee harvest process, this depends on the environment or storage conditions, especially if the coffee beans are not dried according to standards. Factors such as fungal species and strains, water activity, environmental factors (such as climatic conditions, storage and transportation) and processing (wet and dry processing) and storage processes after roasting are factors that are closely related to the presence of OTA contamination in coffee.

4.2. Age of Coffee Consumption and Protein Content in Urine of Coffee Farmers in BPR Ranau Tengah District

It can be stated that in coffee farmers who have the habit of drinking coffee, with increasing age, the risk of protein content in the urine increases. The content of OTA contained in coffee is accumulative and has a half-life of 35 days in the human body so that the habit of drinking coffee in farmers, which mostly starts from the age of toddlers, has a high risk of containing protein in the urine¹⁴.

Generally, farmers with coffee drinking habits require \pm 12 grams (1 tablespoon) of ground coffee with 200 ml of water (1 cup of star fruit) for 1 drink, and consumed as much as \geq 4 cups a day. Consumption of coffee 4-7 cups a day is too much consumption and can pose a risk to health²².

Thus, the habit of drinking coffee for years can cause the accumulation of OTA in the human body, resulting in a high risk of the presence of protein in the urine. OTA can increase collagen secretion which can induce

fibrosis in progressive kidney disease and cause disruption of the kidney's ability to absorb protein²³.

In addition, there is a habit of farmers who store all agricultural products in one room. This is due to the limitations of farmers in providing space for each crop, for example coffee, pepper, palm juice, corn, chili and cabbage are placed in the same room, causing cross-contamination susceptibility between stored agricultural products. Ochratoxin A is not only contained in coffee, but is also found in plant products such as cereals or seeds, spices, vegetable oil, chili and beverages (wine and beer) and even bottled water as well as indirectly through animal feed (grass and concentrate) contaminated with mycotoxins that can leave residues in meat, milk and other products. Therefore, there is a risk of accumulation of contaminants carried from contaminated feed to the tissues and biological fluids of livestock products for human consumption, namely meat, milk and eggs. Usually mycotoxins can enter the human body or livestock through consumption of contaminated food or feed, but inhalation of toxigenic spores and direct skin contact are also important routes of contamination^{24,6,25}.

4.3. Location Association Towards Protein Content in Urine of Coffee Farmers in BPR Ranau Tengah District

The buying and selling system used by coffee farmers in BPR Ranau Tengah District is a barter or exchange system so that the results of coffee processing indicated by OTA contaminants are bartered with coffee processing products that are not indicated by OTA. Coffee farmers exchange dry coffee beans or roasted coffee beans to farmers who have ground coffee because not all farmers have coffee grinders. The locations of the villages where the coffee results indicated the content of ochratoxin A based on microscopic examination were the villages of Tanjung Setia, Suka Marga, Simpang Sender Tengah, Tanjung Kemala and Simpang Sender Timur. Meanwhile, the village

locations that are at risk of suffering from kidney function disorders are Simpang Sender Tengah, Jepara, Tanjung Setia, Serumpun Jaya and Simpang Sender villages. Many conventional farmers' facilities and infrastructure are inadequate, so there are often barter systems or bondage systems in the community¹.

The results of the study stated that, in general, coffee processing carried out by farmers is still conventional with facilities, infrastructure and market access that greatly affect the quality and price of the coffee produced, causing the bondage system and exchange system to apply to the community²⁵.

5. CONCLUSION

There is a significant Association between coffee drinking habits and age with proteinuria content in farmers, there is no significant Association between village location and proteinuria content in farmers in Kec. BPR Ranau Tengah, there are indications of contamination of ochratoxin A in farmers' coffee grounds with the growth of fungi *A. nigri* and *A. ochraceus*.

REFERENCES

- [1] Martauli E.D. 2018. *Analysis Of Coffee Production In Indonesia. Jurnal of Agribisnis science* . April 2018 Volume 01 No 02. 112
- [2] Leitao AL. 2019. *Occurrence of Ochratoxin A in Coffee: Threads and Solutions A-Mini-Review. Beverage* 5 (2): 36. DOI: 10.3390/beverages5020036.
- [3] Lilia D., Nurhayati, Zulkarnain M, Mulawarman., 2021. Short Communication: Drying methods and diversity of contaminant fungi on coffee beans in South Ogan Komering Ulu District, South Sumatra, Indonesia. *Journal of Biodiversity* Volume 22, Number 2, February 2021 Pages: 1037-1042 DOI: 10.13057/biodiv/d220260
- [4] Reichert, B., de Kok, A., Pizzutti, I. R., Scholten, J., Cardoso, C. D., and Spanjer, M. 2018. *Simultaneous Determination Of 117 Pesticides And 30 Mycotoxins In Raw Coffee, Without Clean-Up, By LC-ESI-MS/MS Analysis. Analytica Chimica Acta*, 1004, 40–50. <https://doi.org/10.1016/j.aca.2017.11.077>
- [5] Mata A.T., J.P. Ferreira a, B.R. Oliveira, M.C. Batoréu, M.T. Barreto Crespo, V.J. Pereira. 2015. *Bottled water: Analysis of mycotoxins by LC–MS/MS. M.R. Bronze. Food Chemistry* 176 (2015) 455–464.
- [6] Bucheli, P and Taniwaki M.H. 2002. Research on the origin, and on the impact of post-harvest handling and manufacturing on the presence of ochratoxin A in coffee. https://www.researchgate.net/publication/11264353_Research_on_the_origin_and_on_the_impact_of_post-harvest_handling_and_manufacturing_on_the_presence_of_ochratoxin_A_in_coffee
- [7]. Ferraz, M.B.M.,Adriana F., Beatriz T. Iamanaka., Daniel Perrone, Marina V. Copetti,Viviane X. Marques, Alfredo A. Vitali, Marta H. Taniwaki. Kinetics Of Ochratoxin A Destruction During Coffee Roasting. 0956-7135/\$ - see front matter _ 2009 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodcont.2009.12.001
- [8] Rahim, S.H., Mohd, K.A and Nazarudin, R. 2011. *Fungal Contamination Of Commercial Coffee Powder. 2011(October)*, 24–26.
- [9] Correa P.C., Gabriel H. H. de Oliveira, Ana P. L. R. de Oliveira, Guillermo A. Vargas-Elías, Fábio L. Santos and Fernanda M. Baptestini. 2016. *Preservation Of Roasted And Ground Coffee During Storage Part 1: Moisture Content And Repose Angle. Revista Brasileira De Engenharia Agrícola E Ambiental Campina Grande, PB, UAEA/UFCEG* [http://www. agriambi.com.br](http://www.agriambi.com.br). ISSN 1807-1929. v.20, n.6, p.581-587.
- [10] Martono dan Sartino, 2014
- [11] Hanifah, A. 2012. Pengaruh Penundaan Waktu terhadap Hasil Urinalisis Sedimen Urin.Skripsi. Makasar: Fakultas Farmasi Universitas Hasanuddin
- [12] Wirawan dan Gandasoebrata, 2013. Penuntun Laboratorium Klinis. Jakarta. Dian Rakyat
- [13] Malir F., Vladimir O., Annie P., Jan M and Jakub T. 2016. *Review : Ochratoxin A: 50*

- Years of Research. Toxins* vol 8, 191; doi:10.3390/toxins8070191.
- [14] Houbraken, J., de Vries, R.P., and Samson, R.A. 2014. *Modern Taxonomy Of Biotechnologically Important Aspergillus And Penicillium Species. Advances In Applied Microbiology*, 86, 199-249. Retrieved from <http://dx.doi.org/10.1016/B978-0-12-800262-9.00004-4>
- [15] Khaneghah A.M., Yadolah F., Leili A., 2019. *The concentration and prevalence of ochratoxin A in coffee and coffee-based products: A global systematic review, meta-analysis and meta-regression. Carolina Fernanda Sengling Cebin Coppa, Larissa Tuanny Franco, Carlos Augusto Fernandes de Oliveira. Fungal Biology* 123 (2019) 611-617
- [16] Fuchs R and Peraica, M. 2006. Ochratoxin A in human kidney diseases. DOI:10.1080/02652030500309368
- [17] Desalegn B., Shanika N., Kouji H. H., Toshiaki H., Rohana C., Upul K., Tilak A., Akio K. 2011. *Mycotoxin Detection in Urine Samples from Patients with Chronic Kidney Disease of Uncertain Etiology in Sri Lanka. Bull Environ Contam Toxicol* (2011) 87:6–10. DOI 10.1007/s00128-011-0301-4
- [18] Zaieda C., Chayma B., Islam., Fatma B., 2011. *Presence Of Ochratoxin A In Tunisian Blood Nephropathy Patients. Exposure Level To OTA. Experimental and Toxicologic Pathology* 63 (2011) 613–618. Abdelthif Chourb, Hassen Bachaa, Salwa Abida
- [19] Hope J.H and Hope B.E. 2012. *Review Article: A Review of the Diagnosis and Treatment of Ochratoxin A Inhalational Exposure Associated with Human Illness and Kidney Disease including Focal Segmental Glomerulosclerosis. Volume 2012, Article ID 835059, 10 pages. doi:10.1155/2012/835059. Hindawi Publishing Corporation. Journal of Environmental and Public Health*
- [20] Koszegi T and Poor, M., 2016. Ochratoxin A: Molecular Interactions, Mechanisms of Toxicity and Prevention at the Molecular Level. DOI: [10.3390/toxins8040111](https://doi.org/10.3390/toxins8040111)
- [21] Food Drug and Administration, 2010, Advisory Committee for Pharmaceutical Science. FDA USA
- [22] Schwerdt G., Hildegard H., Christoph S., Maika K., Hans-Ulrich H., Michael G. 2007. *Long-term effects of ochratoxin A on fibrosis and cell death in human proximal tubule or fibroblast cells in primary culture. Toxicology* 232 (2007) 57–67.
- [23] Bhat R, Rai RV, Karim AA. 2010. *Mycotoxins in food and feed: Present status and future concerns. Compr Rev Food Sci Food Saf.* 9:57-81.
- [24] Arroyo-Manzanares N, Huertas-Pérez JF, García-Campaña AM, Gámiz-Gracia L. 2015. *Aflatoxins in animal feeds: A straightforward and cost-effective analytical method. Food Control.* 54:74-78.
- [25] Megeressa B., Getachew W.M., Derese T. 2012. *Original Article: Knowledge and Attitude of Small holder Coffee Producing Farmers to Coffee Quality: The Case of Oromiya and South Nations Nationalities and Peoples Regional States, Ethiopia. Ethiop. J. Appl. Sci. Technol. Vol. 3 (2):* 31-44 (2012)