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FUNGAL INFECTION IN STORED ARABICA COFFEE (*Coffea arabica*) BEANS AT VARIOUS STAGES OF THE DELIVERY CHAIN IN SOUTH SULAWESI PROVINCE

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Running title: Fungal infection in stored arabica coffee beans

ABSTRACT

Indonesia has retained its status as the world's fourth largest coffee producer after Brazil, Vietnam and Columbia, in which one of its well-known coffee is originated from Toraja region, South Sulawesi. Because of this, Indonesia has to compete with these countries in producing good quality coffee beans. The objectives of this research were (a) to obtain information on the postharvest handling methods of Arabica coffee (*C. arabica*) beans in Tana Toraja and North Toraja Regencies, and Makassar Municipality, and (b) to investigate the occurrence of fungi (including ochratoxin A producing fungi) in stored Arabica coffee beans collected from various stages of the delivery chain. Methods used in this study included surveys, interviews and sample collections in each level of delivery chain, which were conducted in May and July 2016. The moisture content and physical quality of the beans were also measured to determine the quality of the beans. The total number of coffee bean samples was 64, consisting of 27 samples from farmers, 15 samples from collectors, 13 samples from traders, and 9 samples from exporters. The results showed that the moisture content of coffee beans collected from farmers and collectors was higher than the maximum tolerable limit determined by SNI (13%), while the moisture content of beans collected from traders and exporters were lower. Based on the total defective value, coffee beans collected from farmers had more diverse grades than those at other levels. *Penicillium citrinum* was the dominat fungus found in coffee beans collected from farmers, collectors and traders, while *Aspergillus niger* was the dominant fungus found in coffee beans collected from exporters. At trader level, 46% of the samples was infected by *Aspergillus ochraceus* and *A. niger*, which are known as OTA-producing fungi. At exporter level, 44% of the samples was infected by *A. ochraceus*, while 78% of the samples was infected by *A. niger*. The postharvest handling methods of Arabica coffee beans conducted especially by farmers and collectors should be improved to minimize moisture content and to increase quality grade of coffee beans.

Keywords: Arabica coffee beans, fungi, moisture content, physical quality

INTRODUCTION

According to Walton (2018) around 70 countries produce coffee, with the overwhelming majority of the supply coming from the developing countries, i.e. Brazil, Vietnam, Colombia, Indonesia and Ethiopia, Indonesia. Therefore, Indonesia is the forth largest coffee beans producer in the world and the second in Southeast Asia after Vietnam. Two kinds of coffee cultivated, i.e. Robusta coffee (*Coffea canephora*) and Arabica coffee (*C. arabica*). GAEKI (2018) reported, that Robusta and Arabica coffee contribute about 83% and 17% of the total coffee production in

Indonesia, respectively. According to Hendrawan (2014) Tana Toraja Regency is one of the Arabica coffee producing regencies in South Sulawesi Province. Tana Toraja coffee is exported to sixteen countries, among others Belgium, the United States, and Japan.

Due to its status as one of the largest coffee producers, Indonesia has to compete with other countries in producing good quality coffee beans. The quality of coffee beans should be examined before they are exported. However, not many people have sufficient skills in tackling the problems during the postharvest handling of coffee beans.

During storage, coffee beans could be infested by insects, microorganisms, mites, and rats. Among microorganisms, fungi are the most dominant cause of deterioration in stored grains or seeds. Fungal infection in grains can cause discoloration, musty odors, weight loss, reduction in nutritional contents, and mycotoxin contamination.

In Indonesia, no research has been conducted on fungal infection (including ochratoxin-producing fungi) in Arabica coffee beans. However, such researches have been conducted in Robusta coffee beans (Dharmaputra *et al*. (2000), Yani (2008) and Nugroho *et al*. (2013).

Ochratoxin A (OTA) contamination in coffee beans have become an important issue recently since some consuming countries are imposing their own maximum tolerable limits (MTL), while the presence of OTA in some coffee products have been reported in several publications. According to Cabañes *et al*. (2010) OTA is a potent nephrotoxic mycotoxin that has been linked to kidney problems in both livestock and human populations. It has also carcinogenic, genotoxic and immunotoxic properties. Natural occurrence of OTA has been reported from temperate to tropical climates mainly on cereals and their products. However, it is also found in a variety of common foods and beverages, including bread, beer, chocolate, coffee, dried fruits, grape juice, pork, poultry and wine among others. Bui-Klimke and Wu (2015) reported that in tropical regions, OTA is mainly produced by *Aspergillus carbonarius*, *A. niger,* and *A. ochraceus*, while in sub-tropical regions it is produced by *Penicillium verrucosum*.

In relation to storage monitoring, this study has two objectives: (1) to obtain information on postharvest handling methods of Arabica coffee beans (*C. arabica*) conducted by farmers, collectors, traders, and exporters and (2) to investigate the degree of fungal infection (including OTA-producing fungi) in stored Arabica coffee beans collected from various stages of the delivery chain in Tana Toraja and North Toraja Regencies, and Makassar Municipality, South Sulawesi Province. The moisture content and physical quality (based on defective value) of coffee beans were also determined because they are known to affect fungal infection.

MATERIALS AND METHODS

Time and Location of Research

Surveys and sampling of Arabica coffee beans were conducted at farmer, collector, trader and exporter levels in Tana Toraja and North Toraja Regencies, and Makassar Municipality, South Sulawesi Province, in May and July 2016. These regencies were selected because they produce large quantities of Arabica coffee beans, while the city of Makassar is the main port for exporting Arabica coffee beans.

Interviews Using Questionnaires

Interviews using questionnaires were conducted during the surveys to collect information on coffee bean postharvest handling methods at different stages of the delivery chain, consisting of farmer, collector, trader, and exporter levels. Data were collected from interviews with respondents. The questionnaires contained questions which were related to postharvest handling methods carried out by farmers, collectors, traders, and exporters, as well as those which were related to problems that they encountered. The number of respondents from each level in the delivery chain was different depending on the number of farmers, collectors, traders and exporters during the surveys (Israel 1992, Science Buddies 2018).

Sampling and Method to Obtain Working Samples

Samples were collected from places where the respondents obtained the coffee beans. The number of Arabica coffee bean samples at each level of delivery chain in Tana Toraja and North Toraja Regencies, and Makassar Municipality, South Sulawesi Province, was larger than that the number of the respondents. Information concerning the number of farmers, collectors and traders was obtained from Toraja Coffee Farmers Cooperative (*Koperasi Petani Kopi Toraja* or KOPTAN PPKT), while that of exporters was obtained from Plant Quarantine Service, Makassar.

Each sample consisted of 1000 g of coffee beans which were collected randomly from each respondent. Each sample was packed in a clean plastic (polyethylene) bag. Several samples were then double-packed in hermetic bags to minimize any changes to the coffee bean samples due to long distance transportation between the location of sampling and the laboratory in Bogor where the samples were analyzed. Each sample was mixed homogenously and then divided two times using a box divider to obtain working samples for the determination of moisture content, quality grade of coffee beans, and fungal population. Samples used to determine fungal population were ground using a grinder.

Determination of Moisture Content, Quality Grade of Coffee Beans, and Fungal Population

The moisture content of coffee beans (based on wet basis) was determined using the oven method (SNI 2008). Two replicates were used for each sample. The quality grade of coffee beans was determined based on the number of defective beans in every 300 g of sample (SNI 2008). Fungi were isolated using the serial dilution method followed by the pour plate method on Dichloran 18% Glycerol Agar (DG18) (Pitt and Hocking 2009). One replicate was used for each sample (1 x 25 g). The fungi were then identified using the method proposed by Pitt and Hocking (2009) as the main reference.

Statistical Analysis

Data consisting of moisture content, total fungal population, and populations of *A. niger* and *A. ochraceus* were analyzed using the non-parametric one-way Kruskal-Wallis test, followed by the Mann-Whitney test.

RESULTS AND DISCUSSION

Postharvest Handling of Arabica Coffee used by Each Level of Delivery Chain

The total number of respondents was 45, consisting of 24 farmers, 10 collectors, 7 traders, and 4 exporters (Table 1). Arabica coffee beans plantations in Tana Toraja and North Toraja Regencies are smallholder plantations. Eight districts, namely Sesean Suloara, Bangkelekila', Tikala, Kapalapitu, Buntu Pepasan, Awan, Rantekarua, Gandasil, and Mengkendek, were the coffee bean producing districts in Toraja Coffee Farmers Cooperative (*Koperasi Petani Kopi Toraja* or KOPTAN PPKT). Four of the eight districts, namely Gandasil, Buntu Pepasan, Kapalapitu, and Sesean Suloara Districts, have large plantations and high levels of Arabica coffee bean production. In 2015 the total areas of coffee plantations in those districts were 538.75, 269.50, 135, and 133.43 ha, respectively, while their levels of coffee bean production were 161,208.4; 53,065.6; 26,369.6; and 24,307.32 kg, respectively. The variety of coffee beans cultivated in these regions was Lini S 795. At the time of our survey, the age of coffee trees ranged between 7 to 31 years old. Coffee trees are intercropped with *lamtoro (Leucaena leucocephala), dammar (Agathis dammara), dadap (Erythrina variegata),* or rubber trees.

Table 1. Number of respondents, sample condition, and number of coffee bean samples collected from various stages of the delivery chain in Tana Toraja and North Toraja Regencies, and Makassar Municipality, South Sulawesi Province.

Level of delivery chain	Number of respondents	Sample conditions	Number of samples
			Total

Farmer	24	HB	27	27
Collector	10	HB	14	15
		GB	1	
Trader	7	HB	2	13
		GB	11	
Exporter	4	GB	9	9
Total	45			64

Notes:
HB (hull beans) = coffee beans with hull
GB (green beans) = dried coffee beans without hull and husk

All our farmer respondents (100%) generally harvested coffee berries in a selective way, i.e. they picked only the ripe ones. All our collector and trader respondents collected coffee beans with hull from farmers, while all our exporter respondents collected green coffee beans (coffee beans without hull and husk) from traders or collectors.

At **farme**r level, the coffee berries were placed in the ex-polypropylene bags. The coffee berries were not processed directly, but they were stored in the polypropylene bags for 1 to 3 days (performed by 42% of respondents), or more than 3 days (performed by 8% of respondents) to obtain enough quantity for the next process. Farmers processed the coffee berries using the wet process. The husks of the berries were shelled, and then the berries were washed using well-water to ensure good fermentation and to eliminate their mucus. Some farmers shelled the husk by treading the coffee berries after being soaked in well-water for two days. The coffee beans were dried using the sun-drying method for less than seven days on dirty polypropylene bags or plaited mats. Consequently, the beans could easily be infected by fungi.

Coffee beans with hulls were then stored in polypropylene bag (performed by 79% of respondents) or using the bulk system (performed by 17% of respondents) for less than seven days. Coffee beans with hulls were sold by the farmers to the collectors. Farmers usually encounter problems during the drying process, because it takes a longer time to dry the coffee beans during rainy season, and this a significant delay decrease the bean quality. They were hoping to get a drying machine from relevant institutions. Moreover, according to our respondent farmers, the price of fertilizer was very expensive, while the price of coffee beans was low.

At **collector** level, the postharvest handling process was conducted faster. Collectors distributed coffee beans with hull directly after they bought them from farmers. They sold the beans to traders, KOPTAN PPKT, or exporters. Seventy percent of our respondents sorted or selected the beans based on grade requirement.

At **trader** level or *Koperasi Petani Kopi Toraja*, coffee beans with hull were re-dried and shelled to produce green coffee, i.e. coffee beans without husk and hull. Traders sorted the beans based on bean size using a grading machine and on-the-beans' defective value manually. Traders

sold those coffee beans among others to exporters. Before selling the beans, they stored the beans for 7 to 60 days. The problems encountered by traders were the lack of roasting equipment and capital.

At **trader and exporter** levels, inappropriate postharvest handling methods were found during the storing of coffee beans because the sanitation was poor and some of them did not use any pallet. Good Postharvest Handling Practice of coffee beans should be based on Permentan (2012). Exporters stored green coffee, i.e. coffee without husk and hull, for six months until two years before they were exported.

Source and Number of Samples

Sample collection was conducted at each respondent's workplace. The total number of coffee bean samples was 64. As many as 27 samples in the form of coffee beans with hull were collected from 24 farmers; 15 samples in the form of coffee beans with hull were collected from 10 collectors; 13 green coffee bean samples, i.e. coffee beans without husk and hull, were collected from 7 traders; and 9 green coffee bean samples, i.e. coffee beans without husk and hull, were collected from 4 exporters.

Each sample consisted of 1000 g of coffee beans which was collected randomly from each respondent. Each sample was packed in a clean polypropylene bag. Before the moisture content, quality grade, and fungal population were measured, the hull of each sample was shelled manually.

Moisture Content and Quality Grade of Coffee Beans

The Indonesian National Standard or SNI has determined the maximum tolerable limit of moisture content (MC) in coffee beans at 13% (SNI 2008). MC of coffee beans collected from farmers (42.5 ± 12.1%) and collectors (42.5 ± 11.3%) were higher than the maximum tolerable limit determined by SNI (13%). As much as 96% and 93% of the samples collected from farmers and collectors had MC exceeding 13% (Table 2). Based on our statistical analysis, different levels of the delivery chain made significant difference in the MC of coffee beans. MC of coffee beans collected from farmers was not significantly different from that of coffee beans collected from collectors. However, MC of coffee beans collected from farmers and collectors were significantly different from those collected from traders and exporters because they were much higher.

Table 2. Moisture content and grade of coffee beans collected from various stages of the delivery chain in Tana Toraja and North Toraja Regencies, and Makassar Municipality, South Sulawesi Province.

Level of delivery chain	Moisture content (% wet basis)	Number (%) of samples with moisture content > 13%	Quality grade (% sample)(*)
Farmer	42.5 ± 12.1 a	26 (96)	1 (11%)
			2 (44%)
			3 (30%)
			4 (7%)
			5 (4%)
			6 (4%)
Collector	42.5 ± 11.3 a	14 (93)	1 (7%)
			2 (53%)
			3 (40%)
Trader	10.9 ± 1.6 b	1 (8)	1 (32%)
			2 (23%)
			3 (15%)
			4 (15%)
			5 (15%)
Exporter	9.7 ± 0.7 c	0	2 (33%)
			4 (45%)
			5 (11%)
			6 (11%)

(*) Source: Indonesian National Standard (SNI 2008)
Number of samples collected from farmers : 27
Number of samples collected from collectors : 15
Number of samples collected from traders : 13
Number of samples collected from exporters : 9

MC of coffee beans collected from traders (10.9 ± 1.6%) and exporters (9.7 ± 0.7%) were lower than the maximum tolerable limit determined by SNI (13%). Nevertheless, 8% of 13 coffee bean samples collected from traders had MC exceeding 13%. Traders and exporters re-dried and re-sorted coffee beans collected from farmers and collectors in order to meet the required standard of quality. Based on the total defective value, the quality grade of coffee beans collected from farmers was the most diverse (grade 1 to 6) than those collected from collectors (grade 1 to 3), traders (grade 1 to 5), and exporters (grade 2 to 6) (Table 2).

Fungal Diversity and Population in Coffee Beans

Based on the results of interviews at various stages of the delivery chain, the respondents said that no fungal problem had ever been found in their coffee beans. Actually, their comments were only based on visual observation. Based on the results of fungal isolation, however, all coffee bean samples were found to be infected by fungi.

Eleven fungal species were isolated in coffee beans collected from **farmers** (Table 3). The most common fungal species which was isolated in 27 coffee bean samples was *Penicillium citrinum* (85%), followed by *Fusarium solani* (26%), *Aspergillus ochraceus* (19%), and *Endomyces fibuliger* (11%). Eleven fungal species were isolated in coffee beans collected from **collectors.** The most common fungal species which was isolated in 15 coffee bean samples was *P. citrinum* (100%), followed by *Cladosporium cladosporioides* (27%), *Endomyces fibuliger* (20%), and *F. verticillioides* (20%). Ten fungal species were isolated in coffee beans collected from **traders**. *Penicillium citrinum* (85%), *A. flavus* (54%), *A. ochraceus* (46%), *A. niger* (46%), *A. tamarii* (38%), and *Eurotium chevalieri* (38%) were the most common fungal species isolated in 13 samples of coffee beans collected from traders. Nine fungal species were isolated in coffee beans collected from **exporters**. *Aspergillus niger* (78%), *A. tamarii* (67%), *P. citrinum* (56%), *A. ochraceus* (56%), and *A. flavus* (44%) were the most common fungal species isolated in 9 samples of coffee beans collected from exporters. From all the species found, *A. niger* and *A. ochraceus* are known to produce OTA.

Leong *et al.* (2007) reported that *A. carbonarius, A. niger,* and yellow Aspergilli (*A. ochraceus* and other species which include *Circumdati* group) were isolated in 65 samples of Robusta coffee beans and 11 samples of Arabica coffee beans in Central Vietnam and South Vietnam. As much as 89% of Robusta coffee bean samples were infected by *Aspergillus niger*, while 12% and 14% of the samples were infected by yellow Aspergilli and *A. carbonarius*, respectively.

Magnoli *et al*. (2008a) reported that 50 samples of Colombian coffee beans (25 green and 25 roasted) were obtained from a processor located in the South of Cordoba province (Argentina). The predominat fungal species found in green coffee samples was belong to *A. niger* agregate, while that of found in roasted coffee was *A. flavus*, followed by *A. niger* aggregate. According to Noonim *et al.* (2008b), 32 samples of dried Arabica coffee beans were collected from two growing sites in the Chiang Mai Province, Thailand. The coffee bean samples collected from the North had an average of 78% incidence of colonization with *Aspergillus* of section *Circumdati* group with *A. westedijkiae* and *A. melleus* as the predominant species. *Aspergillus* spp. of section *Nigri* was found in 75% of the samples, whereas *A. ochraceus* was not found.

281 According to de Fatima *et al*. (2013a) 480 filamentous fungi of the genera *Aspergillus* of the
282 Circumdati and Nigri Sections were isolated from 30 samples of Arabica coffee beans collected
283 from organic (10 samples) and conventional (20 samples) cultivation from the South of Minas
284 Gerais, Brazil. The ochratoxigenic species identified were *A. auricoumus*, *A. ochraceus*, *A.*
285 *ostianus*, *A. niger* and an agregate of *A. niger*. Nugroho *et al.* (2013b) reported that black aspergilli
286 (*A. aculeatus*, *A. carbonarius*, *A. niger* and *A. tubingensis*) were found in 12 samples of dried coffee
287 beans collected from farmers of Kulon Progo District, DIYogyakarta. OTA producing strains were
288 identified as *A. carbonarius* and *A. niger*, meanwhile, *A. tubingensis* and *A. aculeatus* were found as
289 non-OTA producing strains. According to Rezende *et al*. (2013c) as much as 480 filamentous fungi
290 of the genera *Aspergillus* of the Circumdati and Nigri Sections were isolated from 30 samples of
291 Arabica coffee beans collected from organic (10 samples) and conventional (20 samples) cultivation
292 from the South of Minas Gerais, Brazil. The ochratoxigenic species identified were *A. auricoumus*,
293 *A. ochraceus*, *A. ostianus*, *A. niger* and an agregate of *A. niger*. Nganou *et al*. (2014) reported that
294 the important fungi with the potential to produce OTA in Cameroonnian coffee beans are *A.*
295 *carbonarius* and *A. niger*. These two species were predominant on Arabica and Robusta coffee
296 beans.
297 In general there are differences of fungal diversity between OTA producing fungi of
298 Arabica coffee beans in our research and researches conducted by the other authors, except those of
299 *A. niger* and *A. ochraceus*.
300

301 Table 3. Fungal diversity and population in coffee beans collected from various stages of the delivery chain in Tana Toraja and North Toraja
302 Regencies, and Makassar Municipality, South Sulawesi Province

Fungi	Farmer		Collector		Trader		Exporter	
	Number (%) of samples infected by fungi	Range (mean) of fungal population (cfu/g wet basis)	Number (%) of samples infected by fungi	Range (mean) of fungal population (cfu/g wet basis)	Number (%) of samples infected by fungi	Range (mean) of fungal population (cfu/g wet basis)	Number (%) of samples infected by fungi	Range (mean) of fungal population (cfu/g wet basis)
Aspergillus candidus	1 (4)	1.1×10^2 (1.1×10^2)	2 (13)	6.7×10 - 1.3×10^2 (1×10^2)	-	-	-	-
A. flavus	3 (11)	2.3×10 - 1.3×10^2 (7.4×10)	1 (7)	3×10 (3×10)	7 (54)	0.3×10 - 7.7×10^2 (2.4×10^2)	4 (44)	0.7×10 - 1.7×10^2 (5×10)
A. ochraceus	5 (19)	0.7×10 - 5×10^2 (1.4×10^2)	1 (7)	2.3×10^2 (2.3×10^2)	6 (46)	0.3×10 - 2.7×10^3 (7.4×10^2)	4 (44)	0.3×10 - 7×10 (2×10)
A. niger	1 (4)	6.3×10 (6.3×10)	-	-	6 (46)	0.3×10 - 1.3×10^3 (4.8×10^2)	7 (78)	0.3×10 - 8.3×10^2 (3.1×10^2)
A. sydowii	-	-	-	-	1 (8)	1.7×10 (1.7×10)	1 (11)	2×10 (2×10)
A. tamarii	-	-	-	-	5 (38)	0.3×10 - 2.3×10^2 (7.1×10)	6 (67)	0.3×10 - 1.3×10^2 (5.1×10)
A. versicolor	-	-	1 (7)	3.7×10^3 (3.7×10^3)	-	-	2 (22)	0.7×10 - 6×10 (3.4×10)
A. wentii	2 (7)	$(0.3 - 1.7) \times 10$ (1×10)	1 (7)	1×10^2 (1×10^2)	-	-	-	-
Cladosporium cladosporioides	-	-	4 (27)	2×10^2 - 2.3×10^3 (1.4×10^3)	-	-	-	-
Endomyces fibuliger	3 (11)	2.7×10^2 - 1×10^4 (5.1×10^3)	3 (20)	1.7×10^2 - 1.3×10^4 (5.3×10^3)	-	-	-	-
Eurotium chevalieri	3 (11)	1×10 - 1.1×10^3 (4×10^2)	-	-	5 (38)	0.7×10 - 4.7×10^2 (1.2×10^2)	3 (33)	6.3×10 - 1×10^2 (8.2×10)
E. rubrum	1 (4)	4.3×10^2 (4.3×10^2)	2 (13)	3.7×10^2 - 6.3×10^3 (3.4×10^3)	3 (23)	3.3×10 - 1×10^2 (6.7×10)	-	-

Fusarium solani	7 (26)	1.7×10^3 - 8×10^4 (1.9×10^4)	2 (13)	7.7×10^3 - 1.3×10^4 (1.1×10^4)	1 (8)	4×10^3 (4×10^3)	-	-
F. verticillioides	4 (15)	5.3×10 - 4×10^3 (1.2×10^3)	3 (20)	1.3×10^2 - 3.7×10^3 (1.5×10^3)	3 (23)	1×10^2 - 3×10^3 (1.2×10^3)	2 (22)	0.7×10 - 4×10 (2.4×10)
Penicillium citrinum	23 (85)	0.3×10 - 4.3×10^4 (6.3×10^3)	15 (100)	1×10 - 6×10^4 (1.1×10^4)	11 (85)	0.3×10 - 4.6×10^4 (1.2×10^4)	5 (56)	0.7×10 - 3.3×10^2 (1.5×10^2)
Total	27 (100)	0.3×10 - 2.8×10^5 (3.0×10^4)	15 (100)	1×10 - 6.1×10^4 (1.5×10^4)	13 (100)	0.7×10 - 5.4×10^4 (1.3×10^4)	8 (89)	1×10 - 1.1×10^3 (4.9×10^2)

Number of samples collected from farmers : 27
Number of samples collected from collectors : 15
Number of samples collected from traders : 13
Number of samples collected from exporters : 9

Table 3 shows, that population of *A. ochraceus* in coffee beans collected from exporters (2 x 10 cfu/g) was the lowest, compared to those collected from farmers (1.4 x 10^2 cfu/g), collectors (2.3 x 10^2 cfu/g), and traders (7.4 x 10^2 cfu/g). No *A. niger* was found in coffee beans collected from collectors. Population of *A.niger* in coffee beans collected from farmers (6.3 x 10 cfu/g) was the lowest, compared to those collected from exporters (3.1 x 10^2 cfu/g) and traders (4.8 x 10^2 cfu/g). Mean total fungal population in coffee beans collected from farmers, collectors, traders and exporters was 3.0 x 10^4, 1.5 x 10^4, 1.3 x 10^4 and 4.9 x 10^2 cfu/g, respectively. BPOM (2016) has determined the limit of fungal (mould and yeast) population in powder and instant coffees were 10^4 and 10^3 cfu/g, respectively, but no determination has been made on the limit of fungal population in coffee beans.

The high MC of coffee beans collected from farmers and collectors resulted in the high fungal diversity and population in the samples. Traders and exporters re-dried and re-sorted coffee beans collected from farmers and collectors; consequently, the quality of the coffee beans got better i.e. the MC and fungal population were relatively low. The number of defective beans in every sample also affected fungal diversity and their population in the sample. The presence of fungal diversity was probably due to the kind of substrate, i.e. Toraja Arabica coffee beans, and other environmental factors. According to Pitt and Hocking (2009) factors affecting fungal infection in stored foodstuff are water activity, hydrogen ion concentration,temperature – of both processing and storage, gas tension, specifically of oxygen and carbon dioxide, consistency, nutrient status, specific solute effects, and preservatives. Magan *et al*. (2010) reported that fungal diversity found in cereal grain is influenced by abiotic factors such as prevailing temperature and relative humidity, especially at a microclimate level. Thus, the fungi colonizing these ecological niches will interact with each other as they compete to utilize the available nutrients.

Based on our statistical analysis, the delivery chain did not give any significant difference in the total fungal population and population of *A. ochraceus*, but it gave a significant difference in the population of *A. niger* (Table 4). The population of *A. niger* in coffee bean samples collected from traders and exporters was higher than that of samples collected from farmers and collectors.

Table 4. The effect of different stages of the coffee bean delivery chain on total fungal population and populations of *Aspergillus niger* and *A. ochraceus*.

Level of delivery chain	Total fungal population (cfu/g wet basis)	Population of *A. niger* (cfu/g wet basis)	Population of *A. ochraceus* (cfu/g wet basis)
Farmer	3.0 x 10^4 ± 7.2 x 10^4 a	6.3 x 10 a	1.4 x 10^2 ± 1.9 x 10^2 a
Collector	1.5 x 10^4 ± 2.1 x 10^4 a	0 a	2.3 x 10^2 a
Trader	1.3 x 10^4 ± 2.0 x 10^4 a	4.8 x 10^2 ± 5.7 x 10^2 b	7.4 x 10^2 ± 1.5 x 10^3 a

Exporter	$4.9 \times 10^2 \pm 3.9 \times 10^2$ a	$3.1 \times 10^2 \pm 3{,}4 \times 10^2$ b	$2 \times 10 \pm 3.4 \times 10$ a

Note: Means in the same group followed by the same letter in a column are not significantly different at 95%, based on Whitney test.

CONCLUSION

In general, coffee bean samples collected from various stages of the delivery chain were infected by fungi. The percentage of coffee beans infected by OTA producing fungi (*A. niger* and A. ochraceus) were relatively high at trader and exporter levels. The populations of some fungal species exceeded the limit determined by BPOM (2016), while the populations of *A. niger* and *A. ochraceus* were lower than the limit. Fungal diversity and population in coffee beans collected from various stages of the delivery chain were influenced by moisture contens and defective beans; interaction among fungi, and duration of storage. Further research on the possibility of *A. niger* and *A. ochraceus* isolates found in Arabica coffee beans to produce OTA under certain environment should be conducted.

In addition, the postharvest handling method of coffee beans used by farmers, collectors, traders, and exporters in Tana Toraja and North Toraja Regencies, and Makassar Municipality, South Sulawesi Province, should be improved to prevent the possibility of OTA contamination, even though the populations of OTA-producing fungi were found to be lower than the limit determined by SNI. The presence of OTA-producing fungi could serve as an indicator of whether or not Arabica coffee bean samples were contaminated by OTA.

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