Research Paper

Association of Coffee Bean Defects with Ochratoxin A Contamination in the Samples Originated from Jima Woreda of Oromia Regional State, Ethiopia

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Abstract

Filamentous fungi like *Aspergillus*, *Penicillium*, and *Fusarium* are the common microbial contaminants in many agricultural products including coffee beans. These fungal species can produce mycotoxins in coffee beans and exposes consumers to mycotoxin associated health risks. The purpose of this study was to examine the various types of coffee bean defects existed in coffee beans in relation to OTA contamination and search possible physical indicator to identify beans with OTA contamination. Seventy four coffee bean samples were evaluated visually for the presence of defective beans and the defects were counted and scored. The samples were also assessed for fungal incidence and OTA contamination levels. Fungal isolates were identified using species specific primers and rDNA sequencing using ITS1 and ITS4. OTA was analyzed using Enzyme Linked immunesorbent Assay kit. The results showed significant positive correlation (r = 0.56, p < 0.00) between coffee bean defect points and OTA contamination levels. The predominant coffee bean defects encountered in this study in descending order were black beans (41.6 %), immature bean (27.0 %), insect infested (20.5 %), moldy beans (20.4.4 %), and broken beans (15.5%). *Aspergillus ochraceus* and *Aspergillus westerdijikiae* were found to be the predominant ochatoxigenic species encountered in the coffee beans. Ochratoxin A contamination levels in coffee beans were positively correlated with black beans *(r = 0.33, p < 0.03)*, visible mold overgrowth *(r = 0.80, p < 0.00)*, beans damaged by insects *(r = 0.71, p < 0.00)*, and presence of husk *(r = 0.67, p < 0.00)*. Visual inspection of the incidence of these bean defects is a potential indication of OTA contamination in the beans. Therefore, local coffee consumers should avoid coffee beans with sign of mold overgrowth, black beans, beans with husk and damaged by insects as these types of coffee beans are most likely contaminated with OTA and causes health risk.

Keywords: *Black beans, Defect count, Mold, Ochratoxin*

1. Introduction

Coffee consumption in the world was estimated to about 152.1 million bags (60 kg) in 2015 with an average global demand increased by2% (ICO, 2016). Coffee is produced by more than 60 developing countries and the earnings from coffee exports are of vital importance to these countries. Coffee is an important means of social development and a great source of rural employment, providing a livelihood for some 100 million people around the world (Nicolas, 2007; Gray et al., 2013).

Most of the coffee consumed by the local people in Ethiopia is lower quality since some of the beans have been originally destined for export but often rejected from export. Interestingly, even though it may be a lower quality than what is exported, the price of coffee in the local marketplace is sometimes higher than the international price. This high price of substandard

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coffee on the local market has pushed some consumers, particularly those with low purchasing power to utilize even poorer quality beans with very high defects that are characterized by mix of black, sour, broken, discolored, insect damage, moldy, husk, cherry, soil, stick, stone, off odor, Wanza (seed of *Cordia africana*) and others. In worst case this segment of the local community was even forced to make coffee beverage from the husk (skin) of coffee cherry as an alternative to coffee beans (Abu, 2015)

Coffee bean defects are described as foreign materials of non-coffee (e.g., stones/sticks) and non-bean origin (e.g., husks/hulls), abnormal beans regarding shape and visual appearance, such as black beans or any defect that impairs brewed coffee taste and flavor (Leroy et al., 2006). Coffee bean defects can be divided into primary and secondary defects. Black beans and sour beans are usually identified as primary coffee bean defects. Secondary defects principally include broken beans, insect-damaged, faded, green, moldy and silver skinned beans. Bee et al. (2005) and Agresti et al. (2008) have demonstrated that coffee cup quality is strongly compromised by bean defects since they reduce significantly the characteristic quality of coffee beverage. By and large, several kinds of coffee bean defects are encountered in locally sold coffee beans compared to the export portion in coffee growing countries like Ethiopia and Brazil (Mendonca et al., 2008).

Insect infestation of coffee beans can severely reduce both the physical and organoleptic quality of the beans. Moreover, insects have been shown to serve as vectors for a wide array of fungi. In some cases, insect disseminated fungi produce potent toxins in the host plant, which might create sever problems to this agribusiness (Kulandaivelu et al., 2010). Studies indicate that the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) could disseminate toxigenic fungi in coffee plantations (Kulandaivelu et al., 2010). Once the fungi get their way through the cherry, the spores can remain within the holes created by the insect on the beans after harvest and processing. Inappropriate drying and poor storage conditions aid the spores to germinate and increase mycotoxin concentration in the beans (Yan-Xuan et al., 2015).

Beside insect damage, extrinsic coffee bean defects including presence of soil, stone, stick, and husk can serve as means for soil fungi to get into the beans. Based on this background information we hypothesize that coffee beans with higher defect points may contain higher fungal incidence and OTA contamination compared to coffee beans with standard quality. There might be also coffee bean defects that can be used as indicator for OTA contamination in the beans and these defects may serve as possible visual/physical marker for screening OTA contaminated coffee beans during coffee purchase in the local markets. In line with the hypothesis the study aims to evaluate potential physical/visual methods to discriminate OTA contaminated coffee beans that can easily be used by the coffee consumers during raw coffee bean purchase.

2. Materials and Methods

2.1. Coffee Samples

Coffea arabica samples (74) of one kilogram each were collected based on incremental sampling method from farmers' households, market places and mill stores from six woredas in Jimma zone of Oromia regional state from 2014-2015. The samples comprised coffee from different processing methods, varied storage conditions, and having different packaging material. Moisture contents of the coffee samples were recorded at the site of collection using an electronic moisture tester (HOH-Express-HE-50, Germany). The samples were packed in plastic bags, properly labeled, and stored at 4°C for further study.

2.2. Coffee Bean Defect Count

Coffee defect counts were conducted by using the manuals Ethiopian Ministry of Agriculture and Rural Development Coffee and Tea Quality Control and Liquoring Center developed in 2009 (Abrar and Negussie, 2015). From every sample 100 grams coffee beans were taken for defect count. All types of bean defects were counted and defect points were assigned based on their occurrence. If a coffee bean sample has more than one defect, the highest defect is counted. Portion of beans judged as defective were weighed and the weights were converted in to percentage.

2.3. Isolation of Fungi and Infection Prevalence

From each sample, beans were randomly selected and surface sterilized by treating with 1.3% sodium

hypochlorite for 2 minutes. Beans were rinsed three times with sterile distilled water. Malt extract agar medium amended with 0.01% chloramphenicol was used for plating experiments to avoid bacterial outgrowth. Five surface sterilized beans were aseptically placed on a Petri dish and incubated upright at 25°C for 10 days. Every microbial colony that appeared on the beans were counted as black *Aspergilli*, yellow *Aspergilli*, *Penicillium* spp., *Fusarium* spp., and others. Fungal colonies were picked up with a sterile needle and purified by transferring to Czapek's Dox agar medium. Pure isolates were preserved on Czapek's Dox agar slants in the refrigerator at 4°C for further study. The frequency of isolation of fungal genera and group and total microbial load were computed based on the following formula.

$$Frequency\ (\%) \ = \ \frac{NBG}{TN} X\ 100$$

$$Percent\ of\ fungal\ infection\ (\%) \ = \ \frac{NBF}{TN} X\ 100$$

Where NBG represent number of beans from which the genus isolated, NBF is number of beans from which a fungus isolated and TN is total number of beans analyze.

2.4. Fungal Isolation, Morphological and Cultural Examination

Isolates were visually analyzed based on colony growth rates, texture, degree of sporulation, color of mycelia, shape of conidial heads, vesicles, the number of branching points between vesicle and phialides (i.e. uniseriate or biseriate), phialides and conidia were observed for the primary screening of isolates to genus level according to Naviet al. (1999). Representative isolates were maintained in Czapek'sDox agar slant and 10% glyceron in Addis Ababa University and BCCM (Belgian Co-ordinated Collections of Micro-organisms), Belgium respectively.

2.5. Taxonomic Identification of Fungal Isolates

Representative isolates from the genus *Asperillus*, *Penicilliu*, and *Fusarium* were selected for molecular study and mycotoxin analysis. Fungal DNA was extracted from 100 mg of freeze-dried mycelium using cetyl-trimethyl-ammonium bromide (CTAB) protocol as described by Stewart and Via (1993) with some modification. Spores from five day old cultures were inoculated in to yeast extract sucrose (YES) broth

medium on 2mL plastic wells and grown for five days. The mycelia were separated from the broth and transferred to 2 mL epindrhof tubes and maintained at -80°C for one hour. After one hour, the mycelia were freeze dried for an overnight in frieze dryer. The following day the mycelia were pulverized in eppendorf tubes using Micro Pestle. To about 50 mg pulverized mycelia, 500 µL solution containing 1:3(m/v) protinase K in CTAB buffer (2% cetyltrimethylammonium bromide, 1% polyvinyl pyrrolidone,100 mMTris-HCl, 1.4 M NaCl, 20 mM EDTA) was added and vortexed for 30s in a vortex mixer. Then, it was incubated at 65°C for 30 min in a water bath. After incubation the DNA was rested on ice for 15 min. To the cooled NDA 500 µL chloroform isoamyl alcohol (24:1) was added and mixed by rotating the tubes by hand and centrifuged at 13200 rpm for 10 min. The tubes were removed from the centrifuge and 300 µL upper aqueous phases was taken to new tubes. An equal volume (300 µL) of isopropanol was added and mixed gently. Then it was centrifuged at 13200 rpm for 10 min. The supernatant was discarded with micropipette and the pellet was washed with 400 mL of 70% ethanol by centrifugation at 13200 rpm for 1 minute (to remove the isopropanol). After centrifugation, the supernatant was discarded and the pellet was left to dry in the tubes for 15 min in fume hood. After drying, the DNA was resuspended in 50 µL Tris EDTA and maintained at -20°C. DNA concentrations of all samples were determined using Quantusfluorometery, Promega USA according to the machines user manual. Different concentrations ranges were taken and tested for suitability of PCR. Master mix consisting of 14.5µL H_2O (Sigma), buffer 5µL, dNTPs 1.25µL, primers 1µL each (ITS11 and ITS4) and Taq polymerase 0.125 µL was prepared per samples. The master mix (22.85µL) was dispensed in reaction plates and 2µL templet DNA from each sample was added to respective reaction plates. The reaction plates were sealed by a parafilm and all the above procedures were done on ice. PCR products were subjected to gel electrophoresis on 1.5% agarose gel with 1-2µL of loading dye (6X) at 120V for 45 minutes with DNA mass ruler (Thermo Scientific Gene Ruler, 100 bp DNA Ladder). At the end of 45 minutes the gel was submerged in ethidium bromide (0.5µg/mL) for 30 minutes. DNA bands on the gel were captured using Gel

Doc XR+ system integrated with Image Lab™ (BioRad Molecular Imag Gel DecxR, USA).

2.6. Fungal DNA Purification for Sequencing

Fungal DNA was extracted as described before and amplified using ITS1 and ITS4 primers. PCR thermal protocol was as follows: initial 5 min denaturation at 95°C, 32 amplification cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, and a final extension step of 72°C for 10 min in a total volume of 50 µL reaction mix and 2µL temple DNA. PCR products of 5µL were loaded to 1.5% agarose gel with 2µL of 6x DNA loading dye (Life Technologies) and with 100 bp DNA mass ruler (Thermo Scientific) to assess suitability for sequencing. The gel was ran for 45 minutes at 120V and socked in ethidium bromide tank (0.5 µg/mL) for 30 minutes. Images of DNA bands were captured using Bio Rad Molecular Image Gel Dec XR+ (USA) with Image Lab Version 5.0 build 18 software. The samples were purified with the help of E.Z.N.A. Cycle Pure Kit, OMEGA Bio Tek based on the manual provided by the manufacturer. After cold storage for an hour at -80°C the DNA samples were freeze dried for an overnight and sent to sequencing company (Macrogen, Korea). PCR reactions were performed in GeneAmpR PCR system 9700 (Applied Biosystem, Singapore).

2.7. Identification of *Aspergillus* Isolates using Species Specific Primers

Aspergillus isolateswere identifiedusing the primer pairs specific to each species. The PCR conditions for *A. ochraceus* and *A. westerdijkiae* were according to Gil-Semaet al, (2009). For *A. ochraceus*, the PCR cycle comprised 5 min at 95°C, 35 cycles for 30s at 95°C, 30s at 62°C, 40s at 72°C and finally 3 min at 72°C. For *A. westerdijkiae* it was 5 min at 95°C, 30 cycles for 30 s at 95°C, 30s at 63°C, 40s at 72°C and finally 5 min at 72°C. Amplification conditions for *A. niger* was 95°C for 5 min, 35 cycles at 95°C for 30s, 62 to 72°C gradient for 30s, 72°C for 30s and 72°C for 5 min in BioRad T100 Thermal Cycler 2013, Singapore. PCR products were ran on agarose gel and visualized as described before.

2.8. OchratoxinA (OTA) Analysis

For OTA analysis one hundred grams of coffee samples were ground till 50% pass through a 20 mesh screen (0.85 mm) and 10 grams were sub sampled for OTA analysis. The samples (10 gram) were extracted with 80% acetonitrile in distilled water for 5 minutes on a rotary shaker at 150 rpm. The supernatant was filtered with Whatman No.1 filter paper and the clarified extracts were dilute to 10:1 with 70 % methanol in distilled water. OTA analysis was conducted as described in the manual of Sigma Aldrich OTA Elisa Kit (Ref.) for coffee and all the analyses were done in triplicate.

2.8. Data Analysis

For correlation analysis R software version 3.0.3 was used. Spearman's rank correlation was used to examine the association of total bean defect points with OTA concentration levels. Kruskal-Wallis rank sum test was used to evaluate significance differences among the independent variables in relation to the predictor (bean defect point). Multiple comparison tests after Kruskal-Wallis (kruskalmc) was used to see observed values above critical at 0.05 levels. Gen Studio Professional edition was used to process the molecular data before Standard Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov).

3. Results and Discussion

3.1. Results

The result of coffee bean defect count for all samples indicated that 13 types of defects were encountered and all the samples contained defects (Figure 1). The predominant coffee bean defects encountered in this study in descending order were black beans (41.6 %), immature bean (27.0%), insect damaged (20.5%), moldy beans (20.4%), broken beans (15.5%), husk (11.4%) sour beans (7.3%), and cherry (5.9 %)(Figure 1).
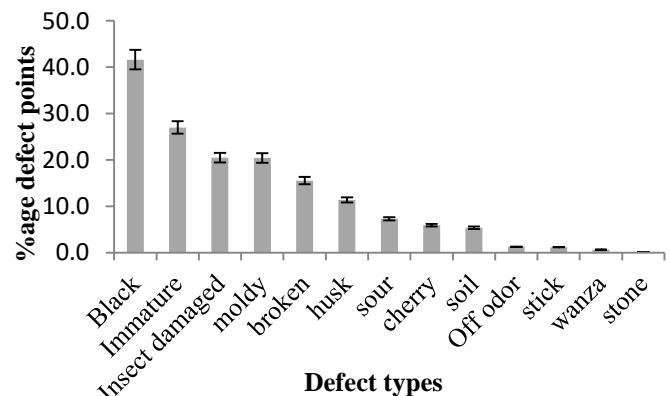

Figure 1: Bean defect types encountered in coffee samples. Error bars are based on 95% confidence interval.

Correlation analysis of coffee bean defect point and OTA concentration in coffee bean samples indicated significant correlation between bean defect point and OTA concentration (r = 0.56, p < 0.00). OTA contamination levels in coffee beans were significantly correlated with black beans (r = 0.33, p < 0.03), visible mold (r = 0.80, p < 0.00), insect damaged beans (r = 0.71, p < 0.00), and husk (r = 0.67, p < 0.00). However, no significance correlations were observed between coffee beans OTA concentration levels and beans with defects including sour (r = 0.16, p < 0.29), presence of cherries (r = 0.15, p < 0.32), sticks (r = 0.00, p < 0.97), soils (r = 0.04, p < 0.81), stones (r = 0.08, p < 0.61), immature beans (r = 0.08, p < 0.59), wanza seed (r = 0.09, p < 0.55), broken beans (r = 0.01, p <0.95) and with off odor beans (r = 0.13, p < 0.38).

3.1.1. Fungal Isolation, Morphological and Cultural Characteristics

More than 500 isolates were obtained from the coffee bean samples and 87% of the coffee samples were contaminated with various fungal species (Figure 2). Most frequently encountered fungal species belonged to the genus *Aspergillus* (79%), *Fusarium* (8%) and *Penicillium* (5%). The remaining 8% belonged to the genera *Trichoderma* and *Rhizopus*spp.
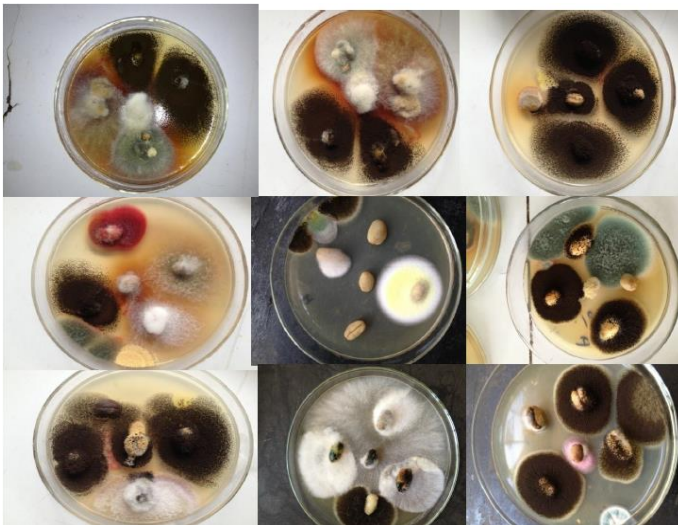

Figure 2: Fungal contamination on coffee samples.

Representative toxigenic fungal pure cultures of *A. westerdijkiae, AochraceuseA. steynii*, and *A. flavus* were indicated in Figures 3 and 4.
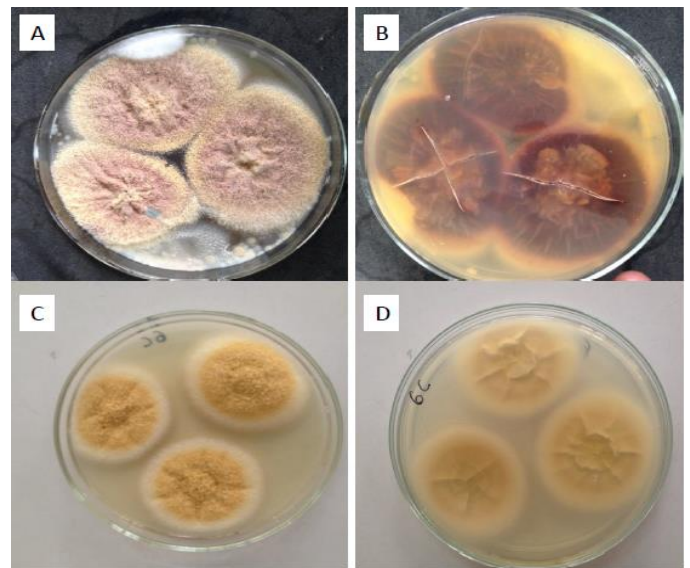

Figure 3: Pure cultures of *A. westerdijkiae* (A = obverse side, B = reverse side) and *A. ochraceus* (C = obverse side, D = reverse side) isolated from coffee beans
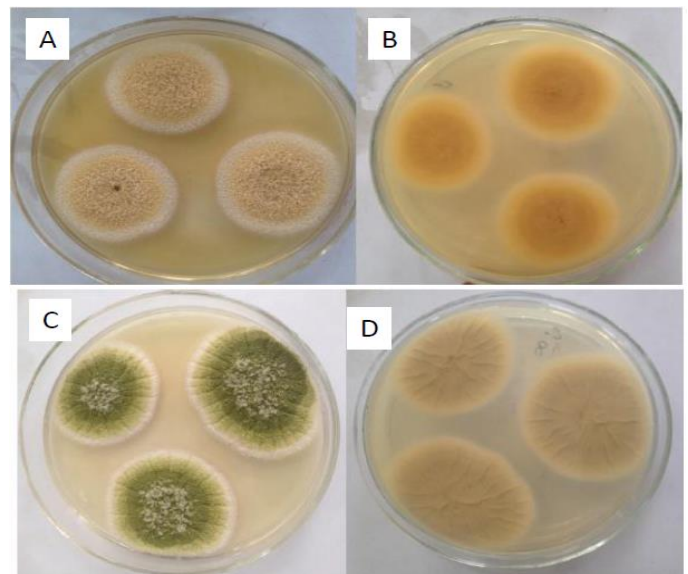

Figure 4: Pure cultres of. *A. steynii (A = obverse side, B = reverse side) and A. flavus (C = obverse side D = reverse side)* isolated form coffee beans

Table 1 shows the sources of coffee bean samples and median values of bean defect, fungal incidence and OTA contamination levels. No significant difference in median bean defect point were observed among the samples collected from different sources (p > 0.23). However, percentage of bean defect point was highest for samples obtained from Addis Ababa (20.40%), followed by Mana (18.04%), Seka (17.54%) and Shebbe (12.99%). The lowest percentage of bean defect was

observed for samples obtained from Jimma town (4.12%) Table 1.

Statistically, no significant difference existed in median coffee bean defect point ($x^2 = 2.78$, $p < 0.43$) with in the processing types. However, median bean defect point obtained for DC coffee samples was highest (94.70), followed by DT (83.80) and WP (69.60) Figure 5.

Table 1. Coffee bean defect points, fungal incidence and OTA contamination levels on coffee samples

Sample Source	Median Beans defect points (%)	Median fungal incidence (%)	Median OTA level (μg/kg)
Dedo (n = 10)	11.16	100	1.77
Gomma (n = 10)	6.90	48	0.12
Shebbe (n = 10)	12.99	96	1.07
Seka (n = 10)	17.54	100	1.18
Limmu (n= 7)	8.82	96	0.42
Mana (n = 11)	18.04	92	2.21
Jimma town (n = 5)	4.12	76	0.87
Adds Ababa (n= 11)	20.40	80	2.21

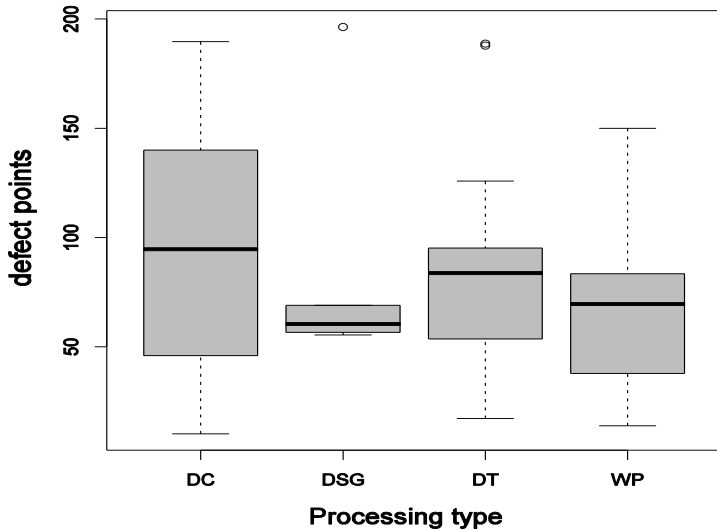NB: n represent number of samples from a specific district.

Figure 5: Coffee bean defect points in relation to processing type (DC = samples dried on concrete DSG = samples dried on soil ground, DT = samples dried by traditional methods like on animal skin, clothes etc., WP < wet processed sample. Box = inter quartile range, bold lines = median values, lower and upper hinges = lower and higher values, circles = outliers).
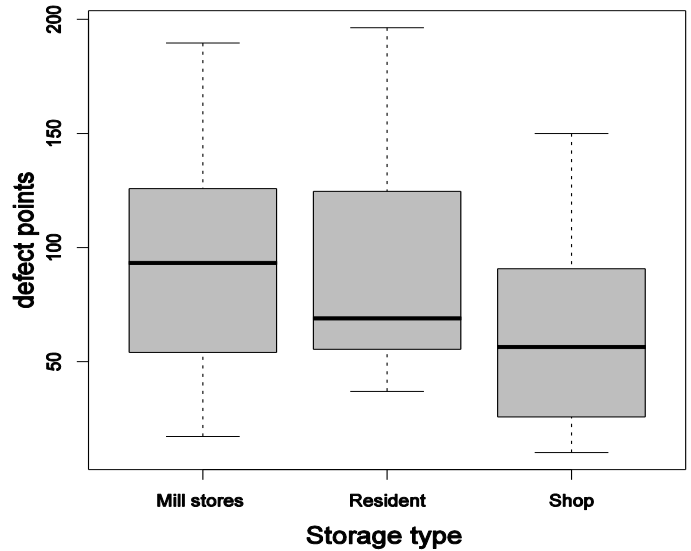

Figure 6: Coffee bean defect points in relation to storage types (Box = inter quartile range, bold lines = median values, lower and upper hinges = lower and higher values).

Significance difference in median bean defect was also observed among the different storage types ($p < 0.00$). Median coffee bean defect point (93) for beans stored in mill store was highest followed by residence (69) and least (56) for bean samples obtained from shop (Figure 6).

No significance difference exists between bean defect points and duration and storage material. There was no correlation between bean defect points and fungal incidences ($r = 0.23$, $p < 0.06$).

3.1.2. Molecular Identification of the Fungal Species

PCR assay using two pairs of species specific primers (OCHRAF, OCHRAR, WESTF and WESTR) enabled the identification of 23 *Aspergillus ochraceus* and 13 *Aspergillus westerdijikiae* species (Figure 7 and 8). DNA sequence analysis using ITS1-ITS4 primers revealed the identity of 25 isolates (2 *A. flavus*, 2 *A. niger*, 1 *A. funigatus*, 1 *A. melleus*, 5 *A. westerdijkiae*, 6 *A. ochraceus* 2 *A. steynii*, 1 *A. tubigenesis*, 1 *G. moniliformis*, 1 *G. zea*, 2 *P. brevicompactum* and 1 *P. verruculosum*). The results of molecular identification indicatedthe majority of fungal isolates belong to the genus *Aspergillus.*
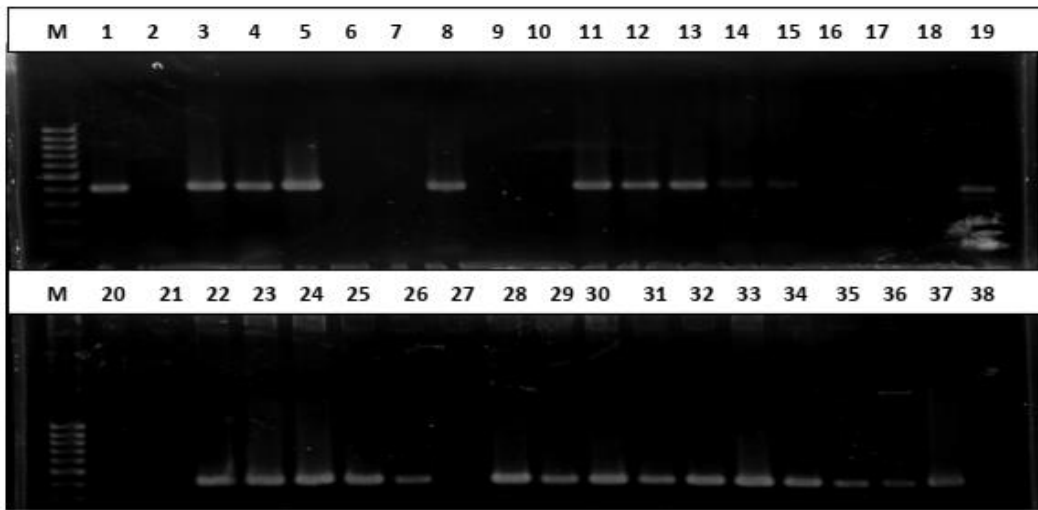
Figure 6: PCR assay with primers OCHRA1 and OCHRA2 specific to *A. ochraceus*

N.B.: Lane M: 1000 pbGeneRuler, Lanes 1-38 samples DNA. Lanes 1, 3-5, 8, 11-15, 19, 22-26, 28-37 indicates DNA of 400 bp identified as *A. ochraceus*.
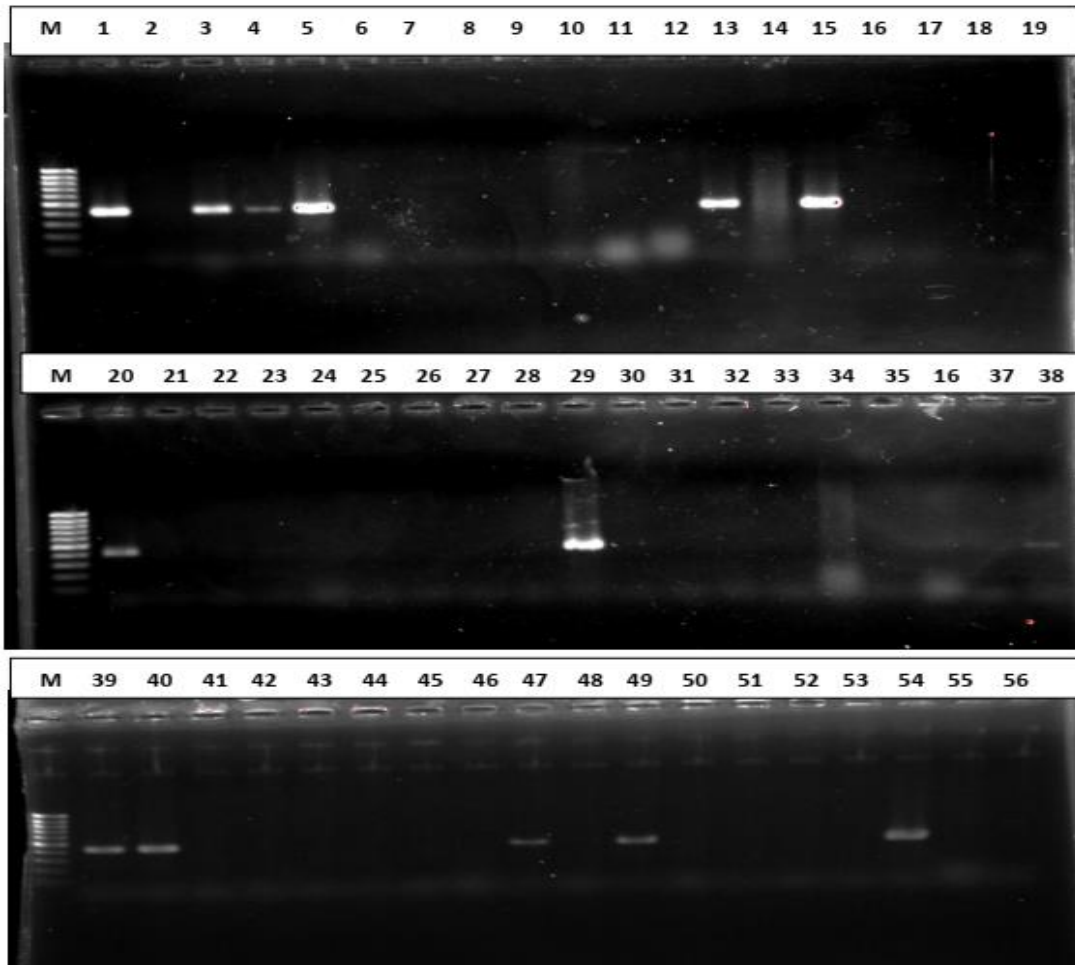

Figure 7: PCR assay with primers WESTF and WESTR specific to *A. westerdijkiae*
N.B.: Lane M: DNA molecular size marker, Lanes 1-56 samples DNA. Lanes no. 1, 3, 4, 5, 13, 15, 20, 29, 39, 40, 47, 49 and 54 indicate DNA of 450 pb, identified as *A. westerdijkiae*

3.2. Discussion

The purpose of this study was to search for possible visual screening method of coffee beans contaminated with OTA based on physical defects so that the local community can easily avoid OTA contaminated beans using the physical appearance of the beans. Coffee beans delivered to local market and consumed by the local community were examined for bean defect levels, bean defect types, fungal incidence and OTA contamination level. The results of the investigation revealed that the average bean defect level was as high as 219 (11 %) in the locally consumed coffee in relation to 86 defects (4%) maximum allowed defect level (SCAA, 2015). Thirteen types of defects were observed in the samples of which four types of defects (black beans, immature beans, insect damaged beans, moldy beans, broken beans and beans mixed with husk) showed significant association with of OTA contamination levels. These defects were identified as the major contributors to higher OTA level in the locally consumed coffee beans. This finding is in line with the study of Taniwaki, et al. (2014) who have investigated that ochratoxigenic fungi and OTA in defective coffee beans and found out the sour and black defective beans had the highest OTA concentration. Similarly, the occurrences of black beans in higher percentage coincide with the finding of Toci and Farah (2008) who have revealed the presence of considerable proportion of black and sour beans in Brazilian Arabica coffee. Several studies (Franca et al., 2005; Pamela et al., 2008) elucidated the difference between defective and non-defective beans in terms of physical and chemical attributes including OTA contamination levels. In search for physical means of discrimination between defective and non-defective beans, Abebe et al. (2014) have reported the presence of higher coffee bean defects in Ethiopian coffee samples that originated from Sidamo (58% defective) and lower for samples obtained from Harar (24.86% defective).

There were two major reasons for the higher proportion of defective beans in the locally sold coffee and portion consumed by the producing farmers. One of the major reasons, as evidently observed during sample collection, was the sorting of sound beans for international market and defective beans for local market. Sound beans were appropriately dried, sorted manually, packed in sisal sacks, and supplied to ECX (Ethiopian Commodity Exchange) for grading. Based on physical defect count and sensorial (organoleptic) analysis grades were given to the beans by trained panel of expertise. Coffee beans failed to satisfy the export standard quality were redirected to the local market. Defective beans removed from quality beans by manual picking were also pooled and mixed with some sound beans and supplied to local markets for the local community. This phenomenon is practiced in the developing countries in the world including the world's top coffee producing country Brazil (Franca et al., 2005).

Secondly, coffee farmers harvest sound red cherries on time and supply to coffee processing firms. Alternatively they can also dry the cherries and supply sound dried cherries to coffee mill houses whose target were foreign market. Any inappropriately harvested, poorly dried, deteriorated cherries collected from soil ground ended up in the nearby open local market at a relatively cheaper price. Even more deteriorated cherries that could not be sold at the local market can be consumed by the families of the producing farmers implying the risk of OTA to the producing farmers.

As explained above, both the coffee processing industries and the coffee farmers contribute to the supply of low quality defective beans to the local market. Unlike cereal grains which can alternatively be used as animal feed, there is no any other option to utilize coffee beans other than beverage making in Ethiopia. As a result, both defective beans rejected by exporters and deteriorated portion supplied to the local market by the producing farmers synergistically contributed to the higher percentage of defects in the locally available coffee. This leads to health risks of local coffee consumers due to consumption of OTA. The relatively lower price of highly defective beans attract very poor portion of the community as this group of community cannot afford to pay for good quality beans at a higher price. This indicates the vulnerability of the poor population group to OTA associated risks in connection with coffee consumption.

Detail explanation of the root causes of all bean defects encountered in this study is beyond the scope of this study. However, in search for the variables accounted for each defect types various processing types, storage types and storage material were evaluated. The statistical analysis of the data did not

support our previous assumption that processing types, storage types and storage materials might have effect on bean defect points. As previously explained by Oliveira et al. (2008) and Taniwaki et al. (2014) bean defects like immature beans, black beans, and sour beans can originate from coffee plantation field related to inappropriate harvesting, and poor processing practices. Therefore, a comprehensive study of coffee processing from field to cup is needed to appropriately point out the root causes of all type of bean defects.

Several authors explained the effect of poor pre and post-harvest practices on the quality of coffee and OTA contamination (Toci and Farah, 2008; Batistaet al., 2009; Birhanet al., 2014).The findings of these authors indicated that bean defects are the result of poor agricultural practices and poor post-harvest handling practices. These poor agricultural practices include inappropriate processing and post-harvest handling practices with wrong storage materials and rooms that contribute to the elevated bean defects and OTA contamination levels.

One of the entrusting finding in this study was the statistically significant association of bean defects including black beans, immature beans, insect damaged beans, moldy beans, broken beans and beans mixed with husk with high OTA contamination level. This finding pointed out the order of significance of these defects to OTA contamination and can be potential indicator of toxin presence in the coffee samples.

The result of OTA analysis indicated the highest toxin level in cherry samples with visible mold overgrowth. As previously hypothesized in this study, visual observation of mold overgrowth on coffee samples implicated with the highest OTA content. This finding is substantiated visually by observable mold overgrowth on coffee samples that can be used as primary evidence for possible OTA contamination in the local market. Since these kinds of coffee are relatively cheaper in price, they attract the poorest communities in the market and this community makes coffee beverage by using both the beans and husk of the cherry. On a previously published paper by Abu and Teddy (2013), the use of coffee husk for coffee beverage making was reported due to the higher price of beans in non-coffee growing areas. The husk of the cherry which is the external cover of the beans is more exposed to fungal and insect attack. Local people who make beverage of

coffee by including the husk are at the highest risk of exposure to OTA. During sample collection for this study it has been observed that the local people were selling and buying the husk. The presence of OTA in coffee husk at a higher proportion was already explained (Taniwaki et al., 2014).

The strong positive correlation of OTA with insect infested beans is another potential indicator of OTA contamination. Insects associated with coffee cherries are known to disseminate toxigenic fungi (Vega et al., 2006) among the cherries and indication of insect puncture on the bean could be one condition for OTA contamination. This finding substantiated the possibility of insect infestation as one clue for OTA contamination. The other potential clue identified in this study for OTA contamination was the presence of husk or the incomplete removal of the husk from the beans. Positive correlation of OTA with husk containing beans implies one of the potential sources of OTA can be the husk. The study of Viani (2002) indicated that coffee husks were significant source of OTA contamination and cleaning of green coffee effectively reduced OTA levels in coffee beans.

The black beans were also found to be associated with OTA in this study. In line with this finding, Taniwaki et al. (2014) have revealed the association of OTA with black and sour defective beans in samples obtained from two coffee growing regions of Brazil. During the sample collection it has been observed that fully black beans were sold at half the price of sound beans (54Ethiopian Birr nearly 2 USD per kg) in Addis Ababa. People purchase these kinds of black beans and according to the sellers these black beans provide bitter taste to coffee beverages and some proportions of the black beans are mixed with quality beans. The commercialization and adulteration of defective beans with sound beans was reported from Brazil (Taniwaki, et al., 2014). However, the supply of defective beans like black, insect damaged, moldy and husk were not reported from Ethiopia. The act of commercializing black defective beans for beverages making in Ethiopian local market apparently contributes to OTA related health risks. This kind of act should be discouraged by setting mycotoxin regulation on the locally sold coffee in order to protect public health. At the same time an alternative way of utilizing the bulk of defective coffee beans that can be removed from export beans should be

explored out instead of damping in the local market for human consumption. Very few reports (Barragan and Rodriguez, 2010) are available on the alternatives use of defective coffee beans as a medium for the growth of pesticide degrading bacteria.

In Ethiopia, local people usually wash the beans or cherry to removes impurities and the off odor from beans before roasting to make coffee beverage. However, washing and roasting cannot remove the toxin and the only option is to avoid beans with an indication of mold overgrowth, insect damage, contains husk and having off odor to scent as these type of beans are associated with the toxin.

The overall fungal contamination obtained in this study (87 %) was nearly similar to results by Fikre*et al.* (2015) (80%) and El Aaraj*et al*. (2015) (87 %) who evaluated fungal incidence from coffee samples obtained from Limmu (Ethiopia) and from Morocco respectively. However, 100 % incidence was reported by Urbano*et al*. (2001) on Robusta coffee samples by the direct plating technique. These findings indicated the poor postharvest handling practices common in developing countries including Ethiopia and it is an area where intervention is needed.

Molecular identification convincingly revealed the presence of various toxigenic fungal isolates associated with coffee samples. Since complete avoidance of toxigenic fungi from agricultural commodity is unavoidable reduction of contamination is possible by implementing good agricultural practices. The detection of OTA in coffee samples substantiates the toxigenic potential of the isolates. However, detail molecular analysis of the fungal isolate was beyond the scope of this paper.

4. Conclusion and Recommendation

This study attempted to search for potential method used by the local community in discriminating OTA contaminated coffee at local market. The result of the study revealed the presence of higher percentage of defective beans in coffee portion supplied to the local market in Ethiopia. The reasons for this higher proportion of defective beans in the locally sold coffee were the supply of off grade coffee failed to pass to international market, mixing of manually sorted defective beans to sound beans, and the preference of farmers to sell good quality coffee to mill stores for export and poor quality to local market.

Presences of higher percentage of defective beans including immature beans, insect damaged beans, moldy beans, beans with husk, and black beans were significantly associated with OTA contamination. These types of coffee bean defects can be used as indicators for possible OTA contamination.

Since defective beans were found to be the potential sources for OTA, coffee processing firms and coffee farmers should avoid mixing defective beans with sound beans to supply to the local market. The defective beans should be rejected as they are hazardous to the health of the consumers. Researchers should explore for an alternative way of utilizing defective coffee beans that are unfit for human consumption.

The local community should make visual and olfactory assessment on the coffee beans and ascertain that the beans should not contains mold overgrowth, infested with insects, mix of husk, musty smell, black beans and mixed cherries. Ethiopian coffee farmers should avoid the drying of cherries on soil ground and picking of cherries fallen on the soil ground for extended period of time in order to avoid or minimize mold colonization. Above ground drying beds and the use sisal sack for storing the cherries are highly recommended.

Reference

Abebe, B., Yirgalem, B., Ataklti, A., Dagne, C. and Hyung, K. (2014). Discrimination of defective (full black, full sour and immature) and non-defective coffee beans by their physical properties. *Journal of Food Process Engineering, 37*, 524 – 532.

Abu Tefera (2015). Ethiopian Coffee Annual Report. USAID GAIN report number ET-1514.

Abu Tefera and Teddy Tefera. (2013). Ethiopian Coffee Annual Report. USAID GAIN report, ET-1303.

Agresti, P., Franca, A., Oliveira, L. and Augusti, R. (2008). Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile. *Food Chemistry, 106*, 787-796.

El-Aaraj, C. E., Bakkali, M., Infantino, A., Arakrak, A., and Laglaoui, A. (2015). Mycotoxigenic Fungi in Cereal grains and coffee from the North of Morocco. *American Journal of Research Communication, 3*, 130-142.

Barragan, B., and Rodriguez, R. (2010). Green bean coffee as nutrient source for pesticide degrading-bacteria. *Current research, Technology and education Topics in Applied Microbiology and Microbial Biotechnology, Mendes, V. (Ed)*, 1322-1327.

Batista, L., Chalfoun, S., Prado, G., Schwan, R. and Wheals, A. (2003). Toxigenic fungi associated with processed (green) coffee beans (Coffea arabica L.). *International Journal of Food Microbiology, 85*, 293–300.

Batista, L., Chalfoun, S., Silva, C., Cirillo, M., Varga, E. and Schwan, R. (2009). Ochratoxin A in coffee beans (Coffeaarabica L.) processed by dry and wet methods. *Food Control, 20*, 784–790.

Bee, S., Brando, C., Brumen, G., Carvalhaes, N., Kolling, I. and Speer, K. (2005). *Espresso coffee, the science of quality*. In A. Illy and R. Viani (Eds.), The Raw Bean (pp. 87–178). Italy: Elsevier Academic Press.

Birhan, T., Musema, A. and Kasahun, M. (2014). Prevalence of Some Coffee Quality Problems in GommaWoreda, Jimma Zone. *World Applied Sciences Journal, 29*, 202-207.

Fikre Lemessa, Amsalu Abera, GirmaAdunga, and Weyesa Garedew. (2015). Association of Mycoflora with Coffee (Coffee arabicaL.) beans at Limmu Coffee Plantation, Southwestern Ethiopia. *Plant Pathology Journal, 14*, 136-141.

Franca, S., Oliveira, S., Mendonc, C. and Silva, A. (2005). Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chemistry, 90*, 89–94.

Gil-Serna, J., Covadonga, V., Noelia, S., Teresa, G. and Belen, P. (2009). Discrimination of the main Ochratoxin A-producing species in Aspergillussection Circumdati by specific PCR assays. *International Journal of Food Microbiology, 136*, 83-87.

Gray, Q., Abu Tefera and Teddy Tefera. (2013). Ethiopian Coffee Annual Report. GAIN Report No. ET 1302.

ICO (2016). International Coffee Organization coffee market report. http://www.ico.org/documents/cy2015-16/cmr-0216-e.pdf accessed August 15, 2018.

Kulandaivelu, V., Rajeev, B. and Thirukonda, N. (2010). Coffee Berry Borer (Hypothenemus hampei) A Vector for Toxigenic Molds and Ochratoxin A Contamination in Coffee Beans. *Foodborne Pathogens and Disease; 7*(10), 1279 -1284.

Leroy, T., Ribeyre, F., Bertrand, B., Charmetant, P., Dufour, M., Montagnon, C., Marraccini, P.and Pot, D. (2006). Genetics of coffee quality. *Brazilian Journal of Plant Physiology, 18*, 229–242.

Mendonca, J., Franca, A., Oliveira, L., Nunes, M. (2008). Chemical characterization of non-defective and defective green Arabica and Robusta coffees by electrospray ionization- mass spectrometry (ESI-MS). *Food Chemistry, 111*, 490–497.

Navi, S., Bandyopadhyay, R., Hall, A., Bramel-Cox, P. (1999). A pictorial guide for the identification mold fungi on sorghum grain. *Information Bulletin, 59*, 22-26.

Nicolas, P. (2007). Ethiopia's Coffee Sector: A Bitter or Better Future? *Journal of Agrarian Change, 7*, 225–263.

Oliveira, L.S., Franca, A.S., Camargos, R.R. and Ferraz, V.P. (2008). Coffee oil as a potential feedstock for biodiesel production. *Bioresource Technology, 99*, 3244-3250.

Pamela, C., Mancha, A., Adriana, S., Leandro, S. and Rodinei, A. (2008). Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile. *Food Chemistry, 106*, 787–796.

SCAA. http://www.coffeeresearch.org/coffee/scaaclass.htm accessed on July 15, 2017.

Stewart, C.N., and Via, L.E. (1993). A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. *BioTechniques, 14*, 748-751.

Taniwaki, M., Teixeira, A., Teixeira, R., Copetti, M., and Iamanaka, B. (2014). Ochratoxigenic fungi and ochratoxinA in defective coffee beans. *Food Research International, 61*, 161–166.

Taniwaki, M.H., Pitt, J.I., Teixeira, A.A., and Iamanaka, B.T. (2003). The source of ochratoxin in Brazilian coffee and its formation in relation to processing methods. *International Journal of Food Microbiology, 82*, 173–179.

Urbano, G. R., Taniwaki, M. H., Leitao, M. F., and Vicentini, M. C. (2001).Occurrence of ochratoxin A producing fungi in raw Brazilian coffee. *Journal of Food Protection, 64*, 1226 – 1230.

Toci, A. and Farah, A. (2008). Volatile compounds as potential defective coffee beans' markers. *Food Chemistry, 108*, 1133 – 1141.

Vega, F., Posada, F., Gianfagna, T., Chaves, F. and Peterson, S. (2006). An insect parasitoid carrying an ochratoxin producing fungus. *Naturwissenschaften, 93*, 297 - 299.

Viani, R. (2002). Effect of processing on OTA content of coffee. *Advances in Experimental Medicine and Biology, 504, 189-93*.

Yan-Xuan, Z., Li, S., Gong-You, Lin, Jian-Zhen, L., Xia, CH., Jie, J., Zhi-Qiang, Zhang, Yutaka, S. (2015). Novel use of predatory mites for dissemination of fungal pathogen for insect biocontrol: The case of Amblyseiusswirskii and Neoseiuluscucumeris (Phytoseiidae) as vectors of Beauveriabassiana against Diaphorinacitri (Psyllidae). *Systematic and Applied Acarology, 20*(2), 177–187