Food Research 3 (6) : 720 - 726 (December 2019)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Prevalence of spoilage mold in coffee before and after brewing

*Kusumaningrum, H.D. and Rasyidah, M.M.

Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Bogor 16680 Indonesia

Article history:
Received: 4 April 2019
Received in revised form: 25 May 2019
Accepted: 28 May 2019
Available Online: 11 June 2019

Keywords:
Commercial ground coffee,
Mold counts,
Spoilage mold,
Traditional brewing

DOI:
https://doi.org/10.26656/fr.2017.3(6).142

Abstract

Commercial ground coffee must be safe for consumption and comply with the regulation applied in a country. However, the risk of the occurrence of spoilage molds in commercial ground coffee, particularly toxigenic mold originated from coffee cherries or green beans, is still a major concern of the coffee industry. This study evaluated the prevalence of spoilage mold in fifteen brands of commercial ground coffee. The spoilage molds were also determined after traditional brewing (non-filtration brewing). The mold counts were enumerated on dichloran-glycerol 18% agar by spread plate method. The spoilage molds were also morphologically identified after isolation on malt extract agar and potato dextrose agar. The results showed that low numbers of molds were found in all samples before brewing, in a range of 10 to 200 CFU/g. A total of eleven genera were identified. *Aspergillus, Cladosporium* and *Penicillium* were found as the predominating genera. After brewing, molds from genera *Alternaria* and *Aspergillus* were still found. However, the total counts decreased to the level between undetected to an average of 3 CFU/mL. This study highlighted that very low levels of spoilage mold was recovered after brewing which may not pose a health risk.

1. Introduction

The quality of green coffee beans can affect the characteristic of coffee beverages and is still a major concern in the coffee industry worldwide. Once the green beans are contaminated, it can decrease the quality and sensory characteristics of the roasted and brewed coffee, giving the unwanted attributes (Iamanaka *et al.,* 2014). The presence of filamentous molds has been already reported in coffee beans processed in Brazil, Malaysia, Philippines, Thailand, and Saudi Arabia (Silva *et al*., 2008; Noonim *et al*., 2008; Alvindia and Acda, 2010; Rahim *et al.,* 2011; Al-Abdalall and Al-Talib, 2012). *Aspergillus, Penicillium, Fusarium* and *Cladosporium* have been found as natural coffee contaminants in Brazil (Silva *et al*., 2008) and present in coffee beans from the field, during fermentation and drying to the warehouse. Alvindia and Acda (2010) also reported that fourteen genera were recovered from coffee beans in the Philippines after harvest and drying. The mold contamination can occur on coffee beans as a result of improper harvesting procedures, inappropriate drying, and inadequate storage conditions. The diversity of the contaminant molds can also be influenced by the region where the coffee beans originated (Noonim *et al*., 2008; Couto *et al.*, 2014).

Although the roasting temperature of coffee beans can eliminate the contaminant mold, however, some spores are not completely eliminated and would be carried over in coffee products. Rahim *et al.* (2011) reported that molds of different genera were still found on eight of twenty commercial black coffee powder samples in Malaysia. *Fusarium* sp. dominated the contamination, followed by *Penicillium* sp., *Aspergillus* sp., and *Cladosporium* spp. Alvindia and Acda (2010) also reported that nine species from five genera were recovered from 21 samples of roasted coffee bean from retail markets in Philippines. *Aspergillus, Penicillium, Chrysosporium, Microascus* and *Rhizopus* were found as coffee contaminants.

Usually, coffee is consumed after hot brewing preparation using a coffee machine or a coffee maker, where filtering is included (Verst *et al*., 2018). However, despite different modern coffee brewing techniques are available, the traditional brewing process is still popular in Indonesia (Sudiyarto *et al*., 2012). This type of coffee known as 'kopi tubruk' or mud coffee in Indonesia is prepared by putting the ground coffee in a cup followed by pouring hot water and held for a few minutes before

*Corresponding author.
Email: *h_kusumaningrum@ipb.ac.id*

eISSN: 2550-2166

serving to let the residues settle down.

Although concerns on the presence of spoilage molds in coffee have been increasing, data on the presence of carried over mold in coffee after brewing have not been widely reported. This study was carried out to determine the presence of mold in coffee before brewing and molds that survive after brewing.

2. Materials and methods

2.1 Samples

A total of fifteen commercial ground coffee samples were purchased from the supermarket around Bogor, Indonesia. The samples consisted of Robusta, Arabica, and a mix of Arabica-Robusta ground coffee. For each sample, a sample size of 100 g was collected and subsampled (25 g) for determination of the mold counts.

From the 100 g of laboratory sample, 10 g of the sample was also weighed and brewed in 150 mL hot water (90^{o}C) without filtering, representing the traditional practice to prepare mud coffee. This mixture was agitated with a spoon for approximately 10 s and then placed at room temperature for 4 mins to settle the residue, prior to the mold determination. For pH measurement, the brewed coffee was further cooled until reaching the room temperature (27± 1°C).

2.2 Enumeration of mold counts

A total of 25 g of ground coffee was aseptically mixed with 225 mL 0.1% buffered peptone water (Oxoid, UK) in a sterile stomacher bag and homogenized in a stomacher (BagMixer 400P, Interscience, France) for 2 mins to provide the first suspension. For brewed coffee after cooled, the first dilution was made by adding of 25 mL beverage solution aseptically to 225 mL 0.1% peptone water and homogenized. Serial dilutions were made under aseptic conditions. For each dilution, 0.1 mL was then pipetted and spread on duplicate plates of solidified dichloran 18% glycerol agar (DG-18, LabM, UK). Plates were incubated at 25^{o}C for 5 days and the colonies were counted and expressed as CFU/g. All samples were analyzed twice.

2.3 pH measurement

The pH of ground coffee was measured using the first suspension, while the pH of the brewed coffee was measured after the coffee was cooled to room temperature.

2.4 Isolation and identification of mold

Any visible mycelia growth or spores from DG-18 plates were transferred onto malt extract agar (MEA, Merck, Germany) and potato dextrose agar (PDA, Oxoid, UK) plates for identification. The plates were incubated at 25^{o}C for 7 days. Isolates were identified on the basis of macro-morphological properties of colonies and micro-morphological properties of conidia and other structures by referring to the key described by Pitt and Hocking (2009).

3. Results and discussion

3.1 Molds counts in coffee before and after brewing

The total molds in commercial ground coffee before brewing were found in a range between 10 CFU/g to 200 CFU/g (Table 1). These loads decreased after brewing, between undetected level (no colony) to an average of 3 CFU/mL. The pH was in a range of 5.12 to 6.12 before brewing and slightly decreased after brewing to between 5.04 to 5.88. The fact that low numbers of mold were still found after brewing in some coffee samples indicated that the spores of some molds were likely resistant to heat treatment during brewing.

The mold counts found in ground coffee before brewing in this study were comparable with the study conducted by Alvindia and Acda (2010) and by Rahim *et al.* (2011). Alvindia and Acda (2010) reported that total mold in roasted bean coffee in Philippines was in a range of 5.3 x 10^{1} to 1.4 x 10^{2} CFU/g. Rahim *et al.* (2011) reported that commercial black coffee powder samples in Malaysia were contaminated by mold in a range of <100 to 1.2 x 10^{3} CFU/g. The presence of mold in roasted ground coffee was influenced by the place of origin of the coffee beans and the processing methods involved. After roasting, the total mold in coffee beans decreased significantly by 93 to 97% (Alvindia and Acda, 2010). The presence of mold after roasting might be due to the post-processing contaminations, the heat resistance of mold spores, or associated with insufficient heat treatment during roasting.

3.2 Mold isolates from coffee before brewing

As shown in Table 2 and 4, more than 250 mold colonies were found on plates from coffee samples before and after brewing. Some molds with similar morphology were found on some plates. From those colonies, sixty isolates were discovered. Some isolates were not identified. Eleven genera were identified on samples before brewing, i.e. *Cladosporium*, *Penicillium, Aspergillus, Alternaria, Geothricum, Phoma, Rhizopus, Chrysonilia, Curvularia, Pestalotiopsis* and *Fusarium* (Table 2). Morphological appearance and growth of some isolates on PDA and MEA plates are presented in Table 3.

The genera *Aspergillus, Penicillium, Cladosporium*,

eISSN: 2550-2166

Table 1. Mold counts and pH of commercial ground coffee and brewed coffee.

No.	Sample Code	Type of Coffee	Mold counts (log CFU/g)		pH	
			Before brewing	After brewing	Before brewing	After brewing
1	KP1	Robusta-Arabica	1.2±0.3[a]	NC	5.80±0.00	5.55±0.01
2	KP2	Robusta	1.5±0.2[a]	NC	6.12±0.01	5.78±0.08
3	KP3	Robusta	1.4±0.6[a]	0.3 ± 0.4	6.05±0.01	5.88±0.01
4	KP4	Robusta-Arabica	1.0±0.0[a]	NC	5.99±0.01	5.73±0.01
5	KP5	Robusta	1.3±0.4[a]	NC	6.03±0.01	5.34±0.01
6	KP6	Robusta	1.2±0.0[a]	NC	5.54±0.00	5.26±0.01
7	KP7	Robusta-Arabica	1.0±0.0[a]	0.5 ± 0.7	5.46±0.00	5.22±0.01
8	KP8	Robusta	2.3±0.2[b]	0.5 ± 0.2	5.63±0.00	5.38±0.00
9	KP9	Robusta	1.5±0.8[ab]	0.2 ± 0.2	5.86±0.01	5.57±0.01
10	KP10	Robusta	1.5±0.3[a]	NC	5.36±0.01	5.30±0.01
11	KP11	Robusta	2.0±0.5[ab]	0.3 ± 0.0	5.53±0.01	5.43±0.00
12	KP12	Arabica	1.3±0.4[a]	0.1 ± 0.1	5.36±0.00	5.17±0.00
13	KP13	Robusta	2.2±0.6[ab]	0.2 ± 0.2	5.40±0.00	5.17±0.00
14	KP14	Robusta	2.2±0.1[b]	NC	5.59±0.00	5.20±0.00
15	KP15	Arabica	2.2±0.0[b]	NC	5.12±0.00	5.04±0.00

NC = no colonies found

Table 2. Identified mold from commercial ground coffee before brewing

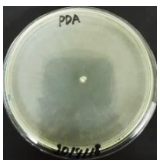
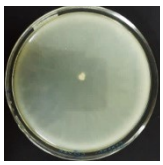
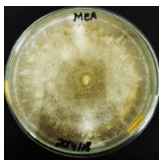
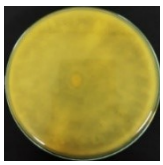
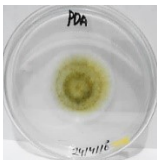
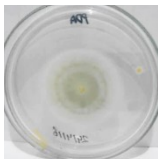
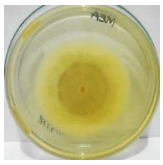
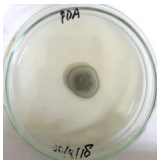
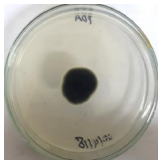
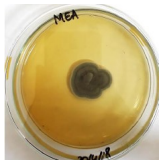
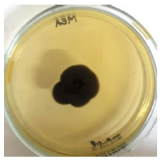
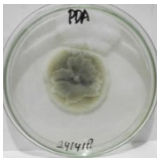
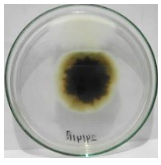
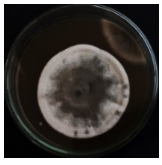
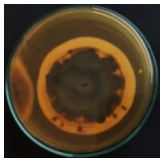
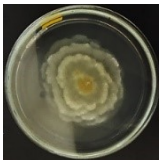
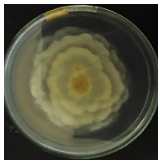
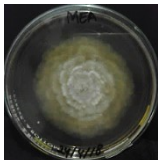
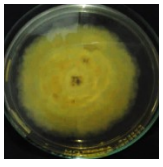
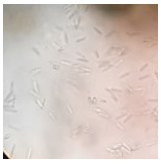
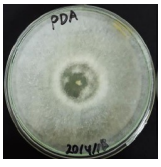
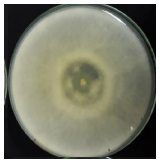
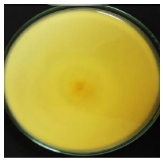
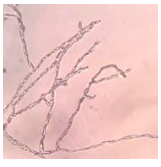
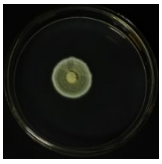
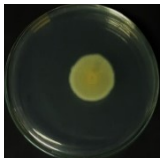
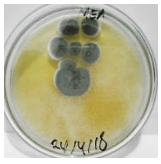
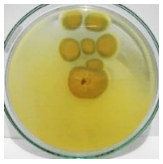
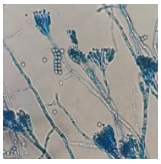
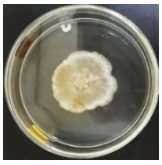
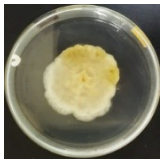
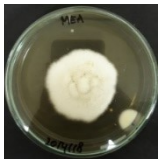
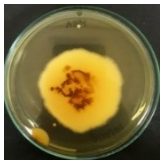
Genera	Species	Isolate	Frequency	Coffee samples
Alternaria	*Alternaria sp. (1)*	12	19	KP 3, 6, 8, 9,10, 12
	Alternaria sp. (2)	27	3	KP 6, 8
Aspergillus	*Aspergillus flavus*	41	57	KP 10, 11, 12
	Aspergillus niger	57	3	KP 10, 14
Cladosporium	*Cladosporium cladosporioides*	15	24	KP 2, 4, 5, 8
	Cladosporium sp. (1)	16	35	KP 5, 8, 10, 13, 14, 15
	Cladosporium sp. (2)	18	2	KP 5, 14
	Cladosporium sp. (3)	24	22	KP 5, 9, 14, 15
	Cladosporium sp. (4)	28	2	KP 7
Chrysonilia	*Chrysonilia sp.*	6	15	KP 2, 11, 12, 15
Curvularia	*Curvularia lunata*	44	6	KP 6, 14
	Curvularia pallesence	45	3	KP 10, 14
Fusarium	*Fusarium sp.*	53	1	KP 15
Geotrichum	*Geotrichum sp. (1)*	1	19	KP 1, 2, 3, 14
	Geotrichum sp. (2)	4	4	KP 1, 9
Pestalotiopsis	*Pastalotiopsis sp.*	21	2	KP 5
Penicillium	*Penicillium citrinum*	51	3	KP 15
	Penicillium corylophilum	50	2	KP 15
	Penicillium islandicum	31	3	KP 8
	Penicillium sp. (1)	17	2	KP 5
	Penicillium sp. (2)	30	4	KP 8, 15
	Penicillium sp. (3)	34	3	KP 9, 14
	Penicillium sp. (4)	48	2	KP14
	Penicillium sp. (5)	49	20	KP 9, 10, 12, 14, 15
Phoma	*Phoma sp.*	13	2	KP 3, 11
Rhizopus	*Rhizopus sp.*	40	2	KP 5, 11

and *Fusarium* were also detected in commercial black coffee powder samples in Malaysia (Rahim *et al.,* 2011). The presence of environmental molds in commercial ground coffee, particularly *Aspergillus, Penicillium,* and *Fusarium* is of the most concern because these molds are known to produce harmful mycotoxins to human. *Aspergillus flavus* was detected in three samples of commercial ground coffee in this study. The presence of *A. flavus* in coffee beans has been reported by Alvindia and Acda (2010) after harvest, drying, and on roasted beans from retail markets. *A. flavus* has also been detected on green coffee during fermentation, drying, and storage in polystyrene and jute sacks (Silva *et al.,* 2008). However, the presence of toxigenic mold in coffee does not always indicate the presence of mycotoxins, since many factors influence the

biosynthesis of mycotoxin. Production of mycotoxin such as aflatoxin is depending on environment condition, particularly water activity and temperature (Mannaa and Kim, 2017).

Furthermore, molds belonging to genera *Fusarium, Pestalotia, Paecelomyces* and *Penicillium* were also detected in coffee cherries, whereas *Fusarium*, *Penicillium* as well as *Aspergillus* were also found in dried coffee beans (Silva *et al.,* 2008). Djossou *et al*. (2015) also reported *that Aspergillus niger, Aspergillus fumigati*, *Penicillium, Fusarium*, and *Mucor* contaminated coffee beans from Ivory Coast. Alvindia and Acda (2010) reported that molds from different genera were found in roasted beans, such as *Aspergillus chevalieri, Aspergillus flavus*, *A. niger, A. fumigatus, Chrysosporium* spp., *Microascus* spp., *Penicillium citrinum, Penicillium janczewskii,* and *Rhizopus oryzae.* Furthermore, Al-Abdalall and Al-Talib (2012) also reported the occurrence of filamentous mold in coffee beans (*Coffea arabica* L.) from grocery stores and retail markets in an eastern region of the Kingdom of Saudi Arabia. The predominating genera were *Aspergillus,* with the highest frequency found was *A. niger* (74.71%). Several molds from other genera were also isolated such as *Fusarium solani* (3.56%), *A. flavus* (2.01%), and *Penicillium oxalicum* (1.61%).

Table 3. Macroscopic and microscopic appearance of mold isolates after 7 days of incubation at 25°C

Morphology on				Microscopy	Genera (Sample)
PDA-above	PDA-bottom	MEA-above	MEA-bottom		
					*Alternaria* (KP3.4)
					*Aspergillus* (KP12.3)
					*Cladosporium* (KP4)
					*Curvularia* (KP12.2)
					*Fusarium* (KP15.4)
					*Geothricum* (KP1.1)
					*Penicillium* (KP 11.4)
					*Pestalotiopsis* (KP5.6)

As shown in Table 2, *Cladosporium* was found as the most often detected on the coffee samples before brewing, i.e. it was found in 10 of 15 samples. *Cladosporium* was also found in commercial black coffee powder samples in Malaysia, although in a lower frequency than *Fusarium* (Rahim *et al*., 2011). *Cladosporium* is known as the most abundant fungi in outdoor and indoor air (Bensch *et al*., 2018). The conidium *Cladosporium* is adaptable in the air because it is small, dry, lightweight, and resistant to sunlight (Pitt and Hocking, 2009). Silva *et al.* (2008) reported that *Cladosporium* was detected in coffee cherries collected from trees, during fermentation and was still found after drying and during storage of dried coffee beans.

In this study, although in low counts, mold from genera *Alternaria* was found in six of ten coffee samples. *Alternaria alternata* (1.2%) was also reported by Al-Abdalall and Al-Talib (2012) in coffee beans from different grocery stores and retail markets. *Alternaria* species are generally found as saprophytes and endophytes of a wide range of plants pre- and post-harvest (Woudenberg *et al*., 2013; Lee *et al.,* 2015). Several taxa, however, were also found as important postharvest pathogens and airborne allergens (Woudenberg *et al*., 2013). In another study, *A. alternata* together with *Cladosporium cladosporioides*, *Pestalotiopsis sp*., *Phoma exigua* var. *exigua* and *Phoma herbarum*, as endophytic fungi, were also isolated from leaves of *Coffea arabica* L. in Brazil (Fernandes *et al*., 2009). The author also pointed out that antibacterial, fungicidal and herbicidal activities were showed by a high proportion of endophytic mold. The culture of *A. alternate* was found as potential antibacterial and antifungal source.

The other molds that were identified in this study were from genera *Chrysonilia, Pestalotiopsis* and *Phoma.* The species *C. sitophila* formerly was known as *Monilia sitophila* (Pitt and Hocking, 2009*).* The genus *Chrysonilia* includes three species, i.e. *C. sitophila, C. crassa* and *C. tetrasperma. Chrysonilia sitophila* has been reported as an inducer of occupational asthma cases in person involved in the coffee industry (Francuz *et al*., 2010). *Pestalotiopsis* isolates were obtained from leaves of *Coffea arabica* in Southern China (Song *et al.,* 2013). *Pestalotiopsis* species is known as weak plant pathogens, but also as common endophytes produces bioactive compounds. *Phoma* is a soil mold that can attack the coffee leaves and cherries. Couto *et al.* (2014) reported that *Phoma* was also found on coffee beans under organic and conventional cultivation. Other genera were also found such as *Aspergillus, Penicillium, Fusarium, Cladosporium, Mucor, Rhizopus, Trichoderma, Colleototrichum, Epicoccum, Bipolaris, Glomerella, Colletotricum, Gliocladium* and *Alternaria.* Organic coffee beans demonstrated greater mold diversity than conventional coffee beans.

3.3. Mold isolates from brewed coffee

Some molds were recovered after the coffee was brewed in hot water at 90^{o}C (Table 4). Some molds were not identified. However, the total molds in brewed coffees decreased to a low level or undetected as presented in Table 1. The temperature applied for coffee brewing in this study was higher than that found in the study of Verst *et al.* (2018). Verst *et al.* (2018) studied the dispensing and serving temperatures of coffee-based hot beverages in the home and in the food service industry. The study reported that of 356 coffees in the food service industry and 110 coffees in private households, the dispensing temperatures were in a range of 58–86°C with an average at 75±5°C.

Table 4. Identified mold from brewed coffee

Sample Code	Type of Coffee	Isolate	Genera
KP3	Robusta	2,3,11, 14	Not identified
		12	*Alternaria sp. (1)*
KP7	Robusta-Arabica	55	*Aspergillus sp. (2)*
KP8	Robusta	3, 11, 14, 39	Not identified
		12,	*Alternaria sp. (1)*
		27	*Alternaria sp. (2)*
		54	*Aspergillus sp. (1)*
KP9	Robusta	2, 3, 14, 39	Not identified
		12	*Alternaria sp. (1)*
KP11	Robusta	3	Unidentified
KP12	Arabica	12	*Alternaria sp. (1)*
		14	Not identified
KP13	Robusta	39	Not identified

As presented in Table 4, *Alternaria* sp. (isolate 12 and 27) were found in five samples, whereas *Aspergillus* sp. (isolate 54 and 55) were found in two samples of brewed coffee. The presence of *Alternaria* sp. in brewed coffee, indicated that they likely produced spores that could be regarded as heat resistant. Ascospore-forming *Aspergillus,* together with *Byssochlamys, Talaromyces*, and *Penicillium* were reported by Pitt and Hocking (2009) belong to the most commonly occurring heat-resistant molds.

In general, the pasteurization process at temperature of 70^{o}C for 10 mins can inactivate *Aspergillus, Fusarium, Penicillium, Mucor* and *Rhizopus* (Yaguchi *et al.,* 2012). However, Jesenská *et al.* (1993) reported that *A. fumigatus*, *Aspergillus nidulans*, *Eupenicillium baarnense* and *Ulocladium spp.* were still recovered after heat treatment at 80°C for 60 mins. The survival of *Acremonium sclerotigenum*, *A. ochraceus, Botryotrichum piluliferum, Byssochlamys fulva, Gilmaniella humicola, Neosartorya fischeri,*

Nodulisporium sp. and *Talaromyces avellaneus* was even still found after heating at 90°C for 10 mins.

Furthermore, the heat resistance of molds from genus *Aspergillus* which were isolated from spoiled pasteurized product has been reported by Berni *et al.* (2017). The D values in glucose solution of *Aspergillus hiratsukae* (≡*Neosartorya hiratsukae*), *A. neoglaber* (≡*Neosartorya glabra*), and *A. thermomutatus* (≡*Neosartorya pseudofischeri*) were in a range between 3.7 to 13.5 mins at 87°C; 1.5 to 3.5 mins at 90°C; and 0.3 to 0.4 mins at 95°C.

4. Conclusion

This study showed that a low level of mold was found in commercial ground coffee before brewing, in a range of 10 to 200 CFU/g. *Cladosporium, Aspergillus and Penicillium* were recovered as prevalent and important genera before brewing. However, the brewing process reduced the mold counts and the diversity of the mycobiota. *Aspergillus* sp. and *Alternaria* sp. were still recovered in very low numbers that expected did not appear to pose a health risk. This study also highlighted that the quality and safety of commercial coffee should be regularly monitored, to obtain and/or maintain safe coffee products for the consumers. Investigating the mycoflora of commercial coffee before and after brewing is worthwhile in providing an overview of the safety of coffee products for consumption.

Conflict of Interest

The authors declared no potential conflict of interest related to the article.

Acknowledgments

The authors thank Ir. Ina Retnowati at Phytopathology Laboratory of SEAMEO BIOTROP Indonesia for her valuable help and technical assistance during identification of mold isolates. The authors also thank the Department of Food Science and Technology, Faculty of Agricultural and Technology, Bogor Agricultural University for facilitating this research.

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eISSN: 2550-2166