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Full Length Research Paper

Fungal Flora of Wheat (Triticum aestivum L.) and Faba bean (Vicia faba L.) Collected from Uraga District, Oromia Region, Ethiopia

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Abstract

Fungal infection of grains' seed could affect the quality and quantity of seeds under storage conditions. Therefore, this study was intended to determine the fungal genera associated with wheat (Triticum aestivum L.) and faba bean (Vicia faba L.) under storage conditions. Agar-plate technique was used to isolate fungal pathogens associated with wheat and faba bean grains samples. In the faba bean, the variability of fungal infections varied from 10.00 to 16/plate and 1 to 7 in the wheat. About 18.18, 27.27, and 27.27% of faba bean seeds were infected with 4, 3, and 2 genera of fungi, respectively. On wheat seeds, the highest fungal infection was found in a sample collected from Suke (SK), followed by Laco Torka (LT) kebele. The number of fungal colonies/samples was the highest in both wheat and faba bean samples of SK kebele, showing a significant difference (P<0.05) from the other kebeles. The relative frequency (RF) of a fungal isolate occurrence was in the range of 1.96 to 19.61% in wheat seeds, with the highest value in SK (19.61%) Kebele. The percentage of seed infection (PSINF) was highest in SK for both wheat (100%) and faba bean (87.00%) with significant variations (P<0.05). In wheat samples, isolates WHHMP4a, WHHP3d, WRBP1b, WRMP4b, and WHRRP3B were identified as Aspergillus spp., and isolates WYBP2b, WLTP3b, and WHSP3a were identified as Fusarium spp. In faba bean samples, FRBP4b, FHWP1a, and FLTP4a were identified as Fusarium spp. In addition, FSKP3c and FYBP4b were identified as the Aspergillus spp. The Chi-square analysis revealed that durations of storage in months (DSIM), durations of storage in years (DSIY), storing materials (SM) used in the home, and the environmental climatic conditions at the harvesting time (ECHT) were the significant risk factors (P<0.05) for fungal infection of wheat and faba bean under storage conditions. Aspergillus and Fusarium were the common fungal genera that infected both faba bean and wheat in the study area. The length of storage and moisture were the two important factors of fungal infection in the grains.

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1. Introduction

Seed-borne pathogens are pathogens such as bacteria, fungi, or viruses that live on the surface or interior of seeds and have the potential to initiate disease in plants under storage and field conditions (Jabir, 2018). Seed health has been considered an attribute

of high quality and one of the most important premises for safe conservation (Farinon *et al.,* 2020). Seed-borne fungal, bacterial, and viral pathogens have a deleterious effect on seeds, through reducing seed viability, vigor, germination capability, shortening longevity of conservation; and causing physiological changes. Furthermore, seed-borne pathogens are also transmitted by seed, which can cause severe diseases in the field (Gebeyaw, 2020).

Fungal infection of grains' seeds probably affects the quality in both direct and indirect manners (Dell'Olmo *et al*., 2023). Direct effects upon seed quality may be due to the growth and ramification of the fungus throughout the kernel and the production of metabolites, which may alter grain composition or metabolism or render it unfit for human or animal consumption. Indirect effects relate to reductions in yield that are associated with contamination. Infected seeds play a considerable role in the establishment of economically important plant diseases in the field, resulting in a heavy reduction of crop yield. Infected seed also has lower seed quality, leading to reduced market value, poor germination, and field establishment (Akranuchat *et al*., 2007).

To date, cereal and legume crops are produced worldwide for their household consumption as a source of nutrients and income. Wheat (*Triticum aestivum* L.) is one of the major cereal crops that are under cultivation, and in the same way, faba bean (*Vicia faba* L.) is widely used to compensate for meat, especially in developing countries. Wheat is the staple food that provides 40% of the world's population with 20% of their food calories (Qamar *et al*., 2021). Faba bean holds great importance for human and animal nutrition for its high protein content. Seeds contain about 29% protein (Warsame *et al*., 2018), and the crop is well adapted to various climates and is grown for both human and animal nutrition (Poysa *et al*., 2006). Wheat and faba beans are the two known crops that are under cultivation and production in the Borena Zone (Basha and Dembi, 2016; Mengistu and Amanu, 2016).

Under storage conditions, fungi are the second biotic factor that affects grains next to insects (Anup *et al*., 2024). Various species of fungi, such as *Aspergillus*, *Penicillium*, *Fusarium,* and *Alternaria,* are the crucial contamination agents of cereal and legume grains (Martín *et al*., 2022). These agents are responsible for both pre-and post-emergence death of grains, affect seedling vigor, and thus cause some reduction in germination and also variation in plant morphology (Karaca *et al.,* 2017). Abubakr (2017) reported *Aspergillus*, *A. flavus*, *A. niger,* and *Fusarium* species as major fungi infecting wheat under storage conditions. In addition, Senbeta and Gure (2014) found that heat seeds were dominated by *Aspergillus* (45.54%) and *Penicillium* (29.18%). *A. flavus*, *A. parasiticus*, *A. niger*, *Penicillium* spp., *Trichoderma* spp., *Mucor* spp., and *Fusarium* spp. were reported to be associated with wheat seeds collected from different areas of Iraq (Ghadban *et al*., 2017). According to Gizaw et al. (2018), *Aspergillus* spp., *Botrytis fabae*, *Uromyces viciae fabae*, *Ascochyta fabae*, and *Fusarium* spp. are the pathogenic fungi associated with faba bean under storage conditions. A study conducted by Senbeta and Gure (2014) in the Shashemene and Arsi Negelle districts indicated a total of 898

fungal isolates belonging to five genera and three unidentified taxa were obtained from the stored wheat samples, where the *Aspergillus* (45.54%) and *Penicillium* (29.18%) were dominant. In addition, *Aspergillus flavus* and *Alternaria triticina* were the predominant species of fungi identified from stored wheat in the southeastern part of Ethiopia, where the highest fungal incidence (98.62%) was recorded after six months of storage (Kesho *et al*., 2020). However, there is limited information on fungal infection of wheat under storage conditions in the Uraga district. Therefore, this study was intended to determine the diversity of fungi associated with selected wheat (*Triticum aestivum* L.) and faba bean (*Vicia faba* L.) seed grains collected from the Guji zone, Uraga district.

2. Materials and Methods

2.1 Description of the study area

Uraga is one of the districts in the Oromia region of Ethiopia that is found at 6°09'60.00" N 38°34'59.99" E. Part of the Guji Zone, Uraga is bordered on the south by Odo Shakiso, on the west by the Borena Zone, on the north by the Southern Nations, Nationalities, and Peoples Region, and on the east by Bore (Figure 1). The 2007 national census reported a total population for this woreda of 176,238 of whom 88, 357 were males and 87, 881 were females; 7, 646 or 4.34 % of its population were urban dwellers. The largest town in the Uraga district is Solomo. Based on the volume of production, major crops grown in the district are maize, wheat, barley, and navy beans. The district's main economic activities are crop and animal production.

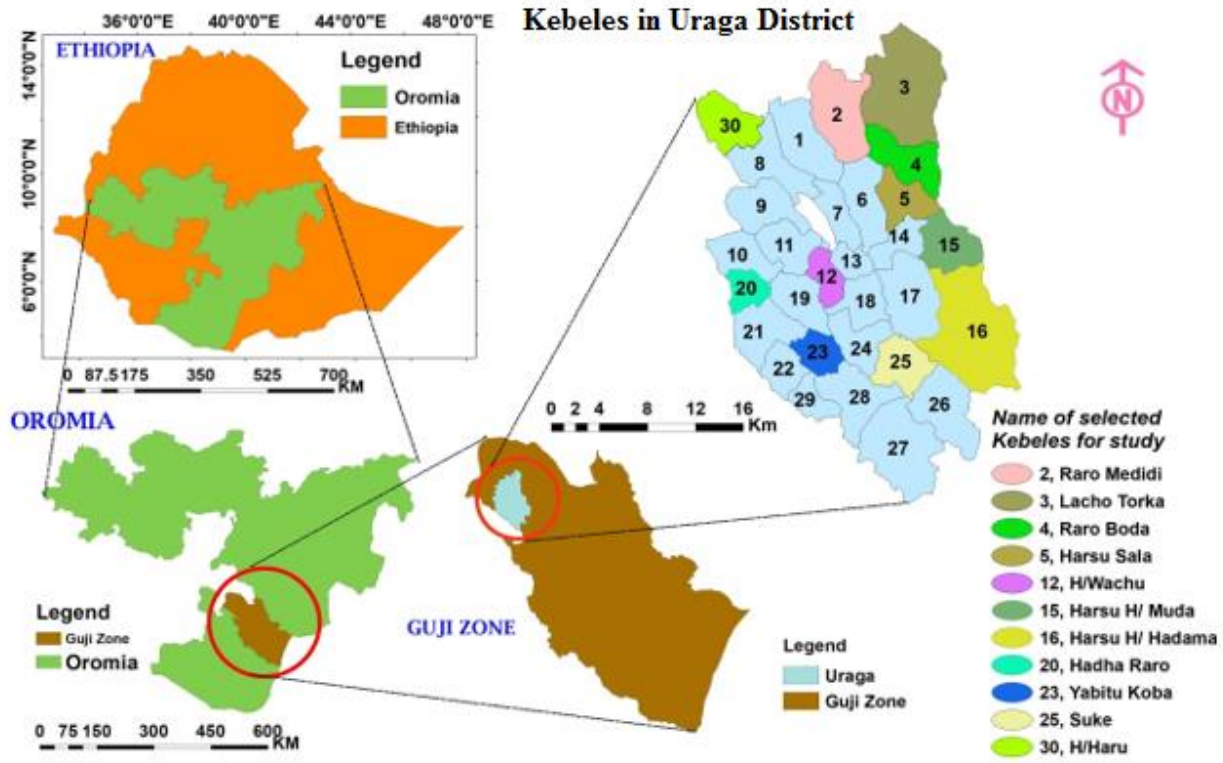

Figure 1. A map showing the study area (district) in Guji zone (constructed by the authors)

2.2 Sampling strategy and the sample size

A stratified random sampling technique was used to collect samples of wheat and faba bean seeds from the study district. There are 30 rural kebeles (i.e., the smallest administrative unit of Ethiopia) in Uraga district, and the samples were collected from each kebele based on accessibility and the presence of surplus-producing farmers. The selection of households (HHs) for sampling in the district was facilitated by key informants from respective kebeles.

2.3 Sample collection and sample preparation

Five replicas of both wheat and faba bean seed samples were collected from each sampling kebele, composited into one sample, and transported to Madda Walabu University for the isolation of seedborne fungi associated with wheat and faba bean seeds. Eleven samples derived from five composites were used for this study, making a total of twenty-two samples. A total of fifty-five (55) wheat seeds were collected from eleven kebeles considering five replicas for each sample, composited to one sample and from each sample for each grain type was collected from eleven Kebeles. Sampling was performed randomly depending on the availability of the sample providers at home and their willingness to give the required grain sample. The collected wheat and faba bean seed samples were surface sterilized by dipping into 1% aqueous sodium hypochlorite solution for 1 min, followed by five successive rinses in sterile distilled water. The samples were blotted dry in between sterile Whatman No. 1 filter paper and plated on Potato Dextrose Agar (PDA) at the rate of 10 grains per plate and incubated at a temperature of 25 ± 2°C for 7 days as described by Hussein and Solomy (2012).

2.4 Isolation and identification of seed-borne fungi

2.4.1 Isolation

Seed-associated fungi were isolated by using the agar plating method with slight modification accordigng to Ramadan and Zrary (2013). The collected seeds were surface sterilized in sodium hypochlorite ($NaClO_2$) for three minutes. Thereafter, seeds were rinsed three times using sterile distilled water and dried on a sterile blotter paper for two minutes. Potato dextrose agar (PDA) medium was used for fungal isolation. Five to six seeds were plated in three replications on the sterile PDA plates and kept at 25$\pm$2 °C for seven days. Seeds were arranged uniformly, making sure that they were placed equidistant from each other on the Petri plate. Subculturing was done using fresh PDA to obtain pure cultures of the fungi.

2.4.2 Identification using microscopy

The process of identifying fungal seed-borne pathogens that formed an overgrowth on the seeds was done using a compound microscope as described by Goko et al. (2021), using slide culture technique. Visual assessment of the presence and characteristics of the fruiting structures was done using mycelial colour formed on the petriplate. The isolated fungi fruiting structures were examined after slide preparation. The seed-borne fungal isolates

were identified through the use of taxonomic features such as conidia and hyphae as described by Hussain et al. (2013) and based on several pictures found in different published articles (Summerell *et al*., 2003; Burgess *et al*., 2008; Wanjiku *et al*., 2020).

2.5 Determination of seeds infection (association) with fungi

The association (infection) of fungi with the targeted seeds was determined according to Kator et al. (2016) using the following formula:

Number of seeds infected (%) =

Number of seeds infected with fungi *100

Total number of seeds per plate

Counts of seeds infected with fungi were recorded based on the type of fungal growth on the seeds. The fungal isolates were distinguished through a visual assessment, where the colors of the colonies were identified by naked eyes and subcultured onto the fresh medium of PDA. The number of fungal colony and genera per sample was calculated by counting the number of colony and genera observed per sample and dividing it to the number of replica and total genera observed, respectively.

2.6 Percentage Occurrence of Fungi

The occurrence of fungi was determined by counting the number of times each individual fungus occurred, divided by the total number of fungi and expressed as a percentage using the following formula (Algabr *et al*., 2018).

Percentage occurrence of fungi=

Number of times each fungus occurred *100

Total number of fungi per plate

2.7 Risk factors for fungal infection

In this study, the questionnaires were used to collect the background information related to the samples collected from the study area. As a result, information such as durations of storage in months (DSIM), durations of storage in years (DSIY), storing materials (SM) used in the home, application of fungicides at field conditions during cultivation (AFDC), the environmental climatic conditions at the harvesting time (ECHT), means of mowing the crop from the field during harvest collection (MMHC), the existence of moisture in the storage places (EMSP), and the existence of fungal disease in the farming areas (EFDFA) was gathered from sample providers during sample collection. The questionnaires were translated into Amharic and Afan Oromo versions as appropriate during sample collection. Questionnaires preparation was done based on different review literature considering factors that are responsible for fungal infection of wheat and faba bean grains under field and storage conditions. The validity and reliability of the questionnaires were checked using Cronbach's coefficient α test (Cronbach, 1951). A Cronbach α value ranging from 0.5 to 0.7 was considered acceptable as indicating the internal reliability of the instrument. A score of > 0.7 was regarded as adequate proof of internal consistency using the following Cronbach's formula:

$$\alpha = \frac{k}{k-1}\left[1 - \frac{\sum_{i=1} pi(1-pi)}{\sigma^2 x}\right]$$

Where α is the Cronbach's coefficient, k is the number of items, pi is the proportion of respondents answering a research question in a certain way, and $\sigma^2 x$ the variance of item i for person y. All the individuals who provided a wheat and faba bean samples were

subjected to responding to the questionnaires.

2.8 Data Analysis

The data obtained from this study were analyzed using one-way analysis of variance (ANOVA) using SPSS version 25, and the Tukey's HSD analysis was employed to separate variation that exists among mean values. The association of risk factors and fungal fungal infection was analyzed using a chi-square (χ^2) test and significance was considered at $p<0.05$.

3. Results and Discussion

3.1 Fungi associated with seed grains of wheat and faba beans

In the present study, the results showed that all the studied samples showed the presence of fungal infections in both the wheat and faba bean seed samples collected from the study district. The pattern of fungal infection showed variations of infections both from place to place and from sample to sample. In wheat, the number of fungal colonies per sample indicated variations of 12.50 to 19, whereas in faba bean, the fungal infections varied from 10.00 to 16 per Petri plate (Table 1). This indicated that the number of fungal colonies was relatively higher in wheat seed samples than observed in the faba bean seeds. In addition, the number of genera per sample varied from 1 to 4 in the faba bean samples of seed and from 1 to 7 in the wheat samples collected from different kebeles found in the study areas. In this perspective, 18.18, 27.27, and 27.27% of faba bean seed samples were infected with 4, 3, and 2 genera of fungi, respectively. Therefore, this study indicated that seed samples of wheat were more prone to fungal infection compared to the seed samples of faba bean collected from selected kebeles of the Uraga district. This may be caused by the physiological requirements of fungal isolates from these two types of seed grains used in the present study (i.e., wheat and faba bean). Thus, the nutritional component of the seed grain samples might have contributed to the variation of fungal infection in the studied seed sample types. Cereals such as wheat are an ideal medium for the growth and multiplication of moulds that are considered a major biotic factor, rendering deceased and/or low-quality cereals (Oliveira *et al*., 2013).

In addition, the storage materials and conditions may have played their own contribution to the variability of fungal infection in both types of seed grains, as farmers always store their grain types separately using different storing materials in different parts of Ethiopia, including the Uraga district. As it was observed in this study, the farmers were found to store their seeds in silos, none plastic and plastic materials (Table 4), of which about 54.5% of the study participants confirmed to store in plastic material. Thus, this might be the basis for variability of fungal infection in the seed samples since the plastic materials are not able to provide aeration and may assist the formation of moisture when stored for a long period of time. In different parts of Africa, smallholder farmers use different storage structures to store their grain. Similarly, in Ethiopia, farmers store their grain in the traditional storage methods that couldn't protect the stored grain from deterioration caused by fungi and pests under storage conditions (Negasa *et al*., 2021).Traditional storage structures found in Ethiopia include:

earthenware pots and gourds, bark, baskets, sacks/bags, basket silos, roof storage, maize cribs, underground pits, small store houses, earthen silos, Gombisa, which is made from split or whole bamboo poles or other tree sticks, and its roof thatched either by dry grass/hay or corrugated iron sheet. These storage conditions may provide opportunity for the different groups of microbial contaminants of seed grains found under the storage conditions.

Table 1: TABLE 1: Fungi isolated from wheat (Triticum aestivum L.) and faba bean (Vicia faba L.) collected from Guji zone Uraga district

Sample code	Area`s Full name	Faba bean (*Vicia faba* L.)		Wheat (*Triticum aestivum* L.)	
		NFC/S	**NFG/S**	**NFC/S**	**NFG/S**
HHM	Harsu Haro Muda	14.75^{ab}	2	15.00^{bc}	3
HH	Harsu Haro Hadama	14.50^{ab}	3	16.50^{ab}	2
SK	Suke	16.00^{a}	4	19.00^{a}	7
RB	Raro Boda	13.50^{bc}	1	17.00^{ab}	3
YB	Yabitu	14.00^{ab}	2	15.00^{bc}	4
HW	Haro Wachu	14.50^{ab}	4	17.00^{ab}	3
LT	Lacho Torka	14.00^{ab}	3	17.25^{ab}	5
RM	Raro Midhidi	10.00^{c}	1	12.75^{c}	1
HHR	Haro Haru	14.75^{ab}	3	15.75^{ab}	3
HRr	Hada Raro	14.00^{ab}	2	15.00^{bc}	3
HS	Harsu Sala	13.25^{bc}	1	12.50^{c}	1
Total	-	**153.25**	**26**	**172.75**	**35**

Keys: NFC/S-Number of fungal colony found per sample, **NFG/S**-Number of fungal genera found per sample. Mean values of four replica represented by the same letter (s) as a superscript in the same column are not significantly different according to Tukey HSD analysis (P>0.05).

Seeds are critical for viable crop production. Pathogen-free seeds are essential for generating healthy plant populations and better harvests (Negasa *et al*., 2021). In many crops, fungal infections are responsible for low-quality seeds. Additionally, the presence of seed-borne pathogenic fungi in beans results in decreased germination, emergence, growth, and yield (Marcenaro and Valkonen, 2016). Based on this point of view, the percentage of seed infection (PSINF) was calculated so as to determine the infection level of the seed samples per sample. The result indicated variation in the percentage of seed infection (PSINF) from sample to sample. In the wheat samples, the PSINF displayed 25.00-100%, whereby the highest seed infection was observed to be in the sample collected from SK (100%), and the lowest infection was in the samples of RM, HHR, and HS (25.00%). In the faba bean seeds, the PSINF indicated 87.00% in SK and 25.00% in HHM samples (Table 2). This may indicate that there are variations between wheat and faba bean for fungal infections under storage conditions, which might be caused by the way in which the fungal infection might be initiated on the seeds used in the current study. During seed production, storage, and transport, the seeds are exposed to many kinds of microorganisms, ultimately resulting in fungal infections that may adversely affect seeds by decreasing germination and vigour, shortening the

storage period, and inducing physiological changes (Baka, 2014). Seeds infected by fungi may survive for 5 years if they are air-dried and stored at 4 °C (Marcenaro and Valkonen, 2016). However, seed quality directly determines the quality of agricultural products. Additionally, seed-borne pathogens can be the primary source of infection and disease transmission.

In the present study, the relative frequency of the fungal isolate was calculated in the wheat and faba bean seed samples collected from several Kebeles of the Uraga district. In the wheat samples, the relative frequency of the fungal isolate occurrence was in the range of 1.96 to 19.61%. Nevertheless, the lowest relative frequency of fungal isolate was noted in the samples collected from RM and HR, which was 1.96% (Table 2). Fungal species composition and percentage of fungal infestation varied among several seeds found under different conditions. Such variations may be attributed to the differences in geographical locality of cultivation, storage conditions, or the differences in physicochemical nature of the different seed samples used in the present study. As indicated in table 2, the relative frequency of fungal occurrence was highest in SK kebele with 19.61%, followed by RB kebele, which showed 13.73% for SKP3d and RBP1b isolates, respectively. In addition, the percentage of seed infection was the highest in SK for both wheat and faba bean, that revealed 100% and 87.00% of seed fungal infection of wheat and faba bean, respectively, with statistically significant variations ($P<0.05$). Moreover, the lowest RF was displayed by isolate YBP2b in Yabitu kebele, followed by RMP4b in Raro Midhidi (RM) and Lacho Torka (LT) kebele.

Table 2: Percentage of seed infection (PSINF) by fungal agents in Wheat (Triticum aestivum L.) and Faba bean (Vicia faba L.) collected from Uraga district and relative frequency (RF) of fungi under storage conditions

	Wheat (*Triticum aestivum* L.)			**Faba bean (*Vicia faba* L.)**		
Sample code	**Isolate code**	**RF (%)**	**PSINF**	**Isolate code**	**RF (%)**	**PSINF**
HHM	HHMP4a	11.76^{ab}	75.00^{bc}	FHHMP1c	1.85^{e}	25.00^{d}
HH	HHP3d	9.80^{bc}	75.00^{bc}	FHHP2a	5.56^{d}	50.00^{cd}
SK	SKP3d	19.61^{a}	100.00^{a}	FSKP3c	11.11^{bc}	87.00^{a}
RB	RBP1b	13.73^{ab}	87.50^{ab}	FRBP4b	11.11^{bc}	65.00^{bc}
YB	YBP2b	9.80^{bc}	50.00^{c}	FYBP4b	16.67^{a}	81.25^{ab}
HW	HWP3b	11.76^{ab}	62.50^{bc}	FHWP1a	5.55^{d}	50.00^{cd}
LT	LTP3b	5.88^{c}	50.00^{c}	FLTP4a	12.96^{bc}	62.50^{bc}
RM	RMP4b	1.96^{e}	25.00^{d}	FRMP3a	14.81^{ab}	62.50^{bc}
HHR	HRP3a	1.96^{e}	25.00^{d}	FHrP1b	5.56^{d}	50.00^{cd}
HRr	HRRP3B	5.88^{c}	50.00^{c}	FHRrP1b	3.70^{de}	50.00^{cd}
HS	HSP3a	3.92d	25.00^{d}	FHSP1a	12.96^{bc}	62.50^{bc}

RF-Relative frequency of fungal genera found dominant in each sample and **PSINF**-Percentage of seed infection for samples collected from each sampling area. Mean values of four replica

represented by the same letter (s) as a superscript in the same column are not significantly different according to Tukey HSD analysis (P>0.05).

Accordingly, the fungi isolated from faba bean samples were grouped into four (4) groups as morphotaxa having more or less similar morphology. In the same manner, the fungi isolated from wheat were categorized into nine (9) morphotaxa. After grouping, the representative culture was re-examined to determine the genera of the isolates listed in the same morphotaxa. Thus, the representative culture from each sampling Kebele, together with their type genera is given below in both wheat and faba bean samples (Table 3).

3.2 Identification of fungal genera associated with wheat seed samples

In wheat samples, isolates WHHMP4a, WHHP3d, WRBP1b, WRMP4b, and WHRRP3B were identified as Aspergillus spp., and isolates WYBP2b, WLTP3b, and WHSP3a were identified as Fusarium spp. (Figure 2). Therefore, the isolates of Aspergillus and Fusarium spp. were the two dominant fungal genera found to infect wheat samples under storage conditions. Among the fungal isolates observed in stored wheat samples, the molds Aspergillus spp. (Aspergillus flavus and A. niger) were the most predominant external mycoflora (Dawood and Elshamry, 2015).

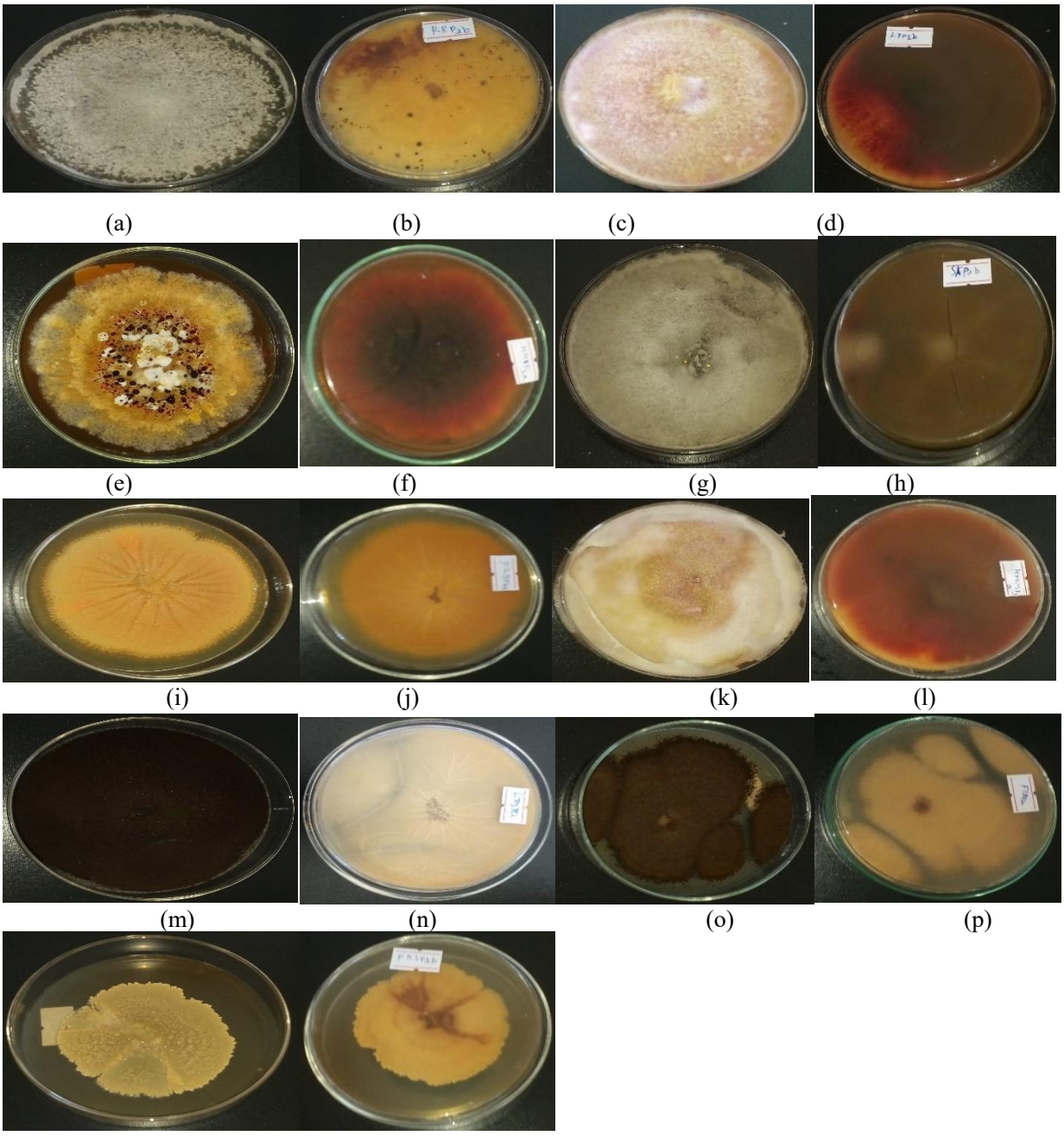

Figure 2. Representative fungal isolates obtained from wheat (*Triticum aestivum* L.) samples with their front and reverse view. a) *Cladosporium* sp. (front view), (b) *Cladosporium* sp. (reverse view), (c) *Fusarium* sp. (front view), (d) *Fusarium* sp. (reverse view), (e) *Phoma* sp. (front view), (f) *Phoma* sp. (reverse view), (g) *Fusarium* sp. (front view),(h) *Fusarium* sp. (reverse view),(i) *Aspergillus* sp. (front view),(j) *Aspergillus* sp. (reverse view),(k) *Fusarium* sp. (front view),(l) *Fusarium* sp. (reverse view),(m) *Aspergillus* sp. (front view),(n) *Aspergillus* sp. (reverse view),(o) *Aspergillus* sp. (front view),(p) *Aspergillus* sp. (reverse view),(q) *Alternaria* sp. (front view) and (r) *Alternaria* sp. (reverse view).

3.3 Identification of fungal genera associated with faba bean seed samples

In faba bean samples, FRBP4b, FHWP1a, and FLTP4a were identified as *Fusarium* spp. In addition, FSKP3c and FYBP4b were identified as the *Aspergillus* spp. (Figure 3). Therefore, the present study revealed that the genera of *Aspergillus* and *Fusarium* were the two common genera that infect faba bean and wheat, with the states of one and two ranks in wheat and faba bean, respectively. This result agreed with that reported by Barros et al. (2005) and Bashir et al. (2013), who stated that there was a high degree of mould contamination in stored grains. Interestingly, the potent fungi that act as the biological control agents of several phytopathogens, *Trichoderma* spp. (i.e., isolate FRMP1a), were found in faba bean samples. However, one isolate (i.e., isolate FHSP1a) was not able to be identified, which may need further characterization in order to determine its genus and/or species. According to Alkenz et al. (2015), research work to explore fungi and mycotoxins associated with rice grains during storage for 25 rice samples collected from different locations of district Mandi in India revealed that all the samples were found to be contaminated with one or more fungal genera of *Aspergillus* (41.6%), *Fusarium* (8.3%), *Penicillium* (16.6%), and other genera (41.5%).

Table 3: Fungal groups associated with Wheat (Triticum aestivum L.) and Faba bean (Vicia faba L.) under storage conditions

Wheat (*Triticum aestivum* L.)			**Faba bean (*Vicia faba* L.)**	
Sample code	**Isolate code**	**Genera identified**	**Isolate code**	**Genera identified**
HHM	WHHMP4a	*Aspergillus* sp.	FHHMP1c	*Penicillium* sp.
HH	WHHP3d	*Aspergillus* sp.	FYBP4c	*Trichoderma* sp.
SK	HHSKP3d	*Phoma* sp.	FSKP3c	*Aspergillus* sp.
RB	WRBP1b	*Aspergillus* sp.	FRBP4b	*Fusarium* sp.
YB	WYBP2b	*Fusarium* sp.	FYBP4b	*Aspergillus* sp.
HW	WHWP3b	*Cladosporium* sp.	FHWP1a	*Fusarium* sp.
LT	WLTP3b	*Fusarium* sp.	FLTP4a	*Fusarium* sp.
RM	WRMP4b	*Aspergillus* sp.	FRMP1a	*Trichoderma* sp.
HHR	WHRP3a	*Alternaria* sp.	FHSP1a	*Rhizopus* sp.
HRr	WHRRP3B	*Aspergillus* sp.	FHRrP1b	*Cladosporium* sp.
HS	WHSP3a	*Fusarium* sp.	FHHP2b	*Chaetomium* sp.

"**W**" and "**F**" in the above table under isolate code stands for Wheat (*Triticum aestivum* L.) and Faba bean *(Vicia faba* L.) respectively being together with the names of Kebeles, P1, P2, P3, and P4 stands for the plate number. In addition, a "Lower Case" letters (a, b, c, and d) indicate the category of the isolates found per plate.

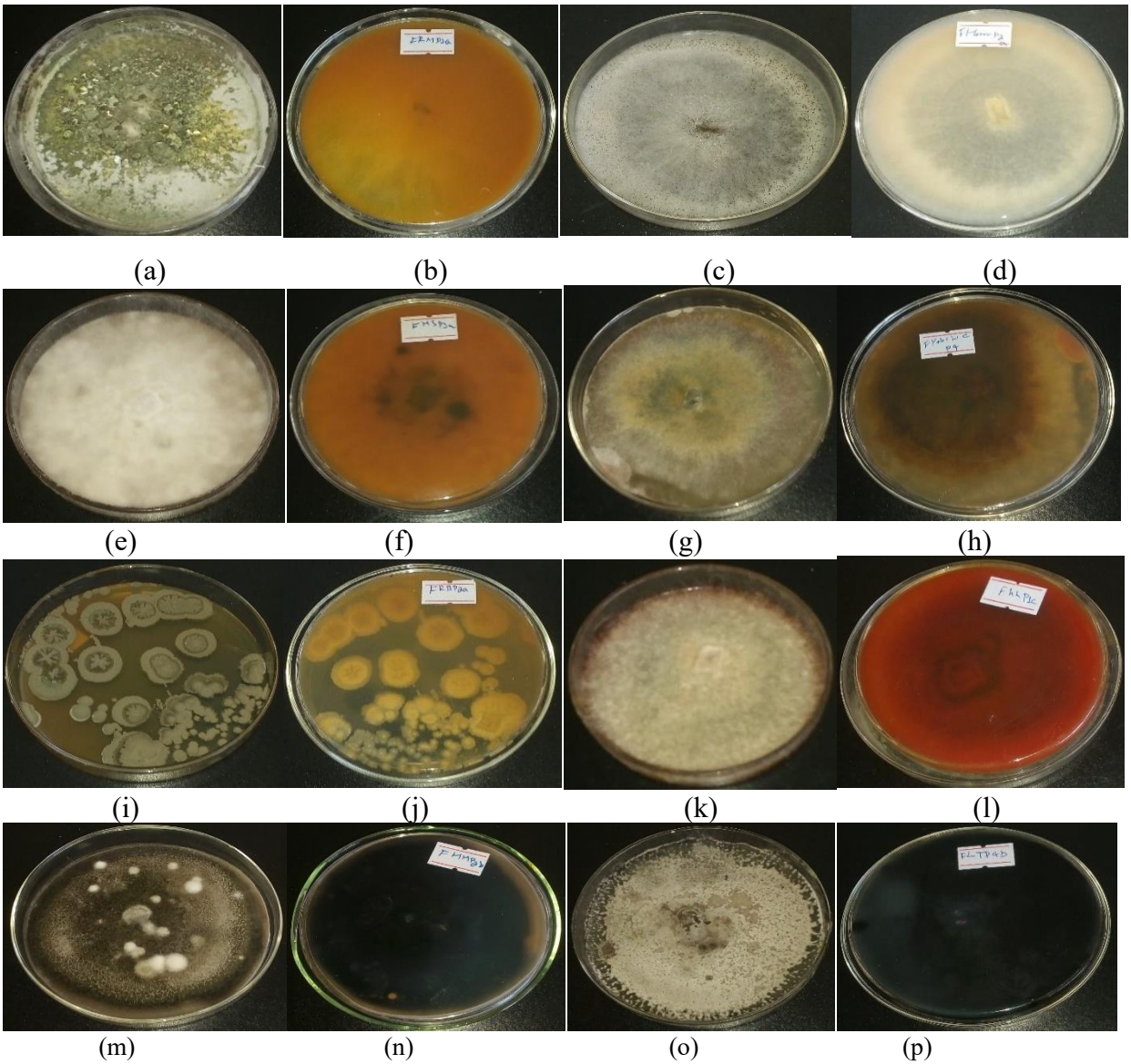

Figure 3. Representative fungal isolates obtained from faba bean (*Vicia faba* L.) with their front and reverse view. (a) *Trichoderma* sp. (front view), (b) *Trichoderma* sp. (reverse view), (c) *Fusarium* sp. (front view), (d) *Fusarium* sp. (reverse view), (e) *Rhizopus* sp. (front view), (f) *Rhizopus* sp. (reverse view), (g) *Trichoderma* sp. (front view), (h) *Trichoderma* sp. (reverse view), (i) *Penicillium* sp. (front view), (j) *Penicillium* sp. (reverse view), (k) *Fusarium* sp. (front view), (l) *Fusarium* sp. (reverse view), (m) *Chaetomium* sp. (front view), (n) *Chaetomium* sp. (reverse view), (o) *Cladosporium* sp. (front view) and (p) *Cladosporium* sp. (reverse view).

3.4 Contributing factors of fungal infection in faba bean and wheat samples

The Chi-square analysis revealed that durations of storage in months (DSIM), durations of storage in years (DSIY), storing materials (SM) used in the home, and the

environmental climatic conditions at the harvesting time (ECHT) were the significant risk factors ($P<0.05$) for fungal infection of wheat and faba bean under storage conditions (Table 4). All the respondents said about 100% of the seeds collected from Suke (SK) kebele were stored for more than 12 months, and it was 3 years in duration under storage as 91.0% of the respondents said. As a result, the highest number of fungal colonies was found in both wheat and faba bean samples collected from SK. In addition, 82% of the sample providers confirmed the existence of moisture in the storage places of the samples taken from SK Kebele. Storage fungi are among the major factors causing post-harvest deterioration of crop produce worldwide when the storage time is long in a place that has a relatively high moisture level (Ren *et al*., 2020). Thus, the length of storage and moisture can be considered as the two important parameters of fungal infection of seed grains under storage. According to Kesho et al. (2020), fungal contamination of wheat seeds varied with storage period, with the highest incidence of 98.62% followed by 89.78% and 86.77% after six months, upon harvest, and three months of storage, respectively. As the study indicated, the highest fungal incidence (98.62%) was recorded after six months' storage of the grain. Fungal incidence was highly associated with two of the independent variables, namely, temperature and relative humidity of storage.

In Raro Boda (RB) Kebele, the sample was known to be stored in plastic sacks; in Yabitu (YB) Kebele, there was the existence of fungal disease in the farming areas, as about 72.7% of the respondents stated. In Haro Wachu (HW) Kebele, 72.7% of the respondents stated the presence of moisture in their seed storage places at home. In Lacho Torka (LT), 72.7% of seeds were found to be stored for more than three years. In Raro Midhidi (RM), 72.7% stated no application of fungicides at field conditions during cultivation. However, about 82% of the study participants revealed the seed storage for about 3 years and no application of fungicides under field conditions during cultivation of the crops in Haro Haru (HHr) Kebele. Therefore, this may indicate the role of storing material in the prevention of fungal infection and the use of fungicides during cultivation, which may avoid the introduction of fungi from farmland to the storage place after harvesting the crops.

In Hada Raro (HRr) Kebele, 91.0% of the respondents said seed storage for more than a year, no application of fungicides under field conditions during cultivation of the crops, the presence of clouds during sample collection, the availability of moisture in the storage place, and the existence of fungal disease in their farming areas. In Harsu Sala (HS) Kebele, 82% of the sample providers said the storage of their seeds for three years and the existence of moisture in their seed storage places.

In SK kebele, about 82 of the sample providers did not apply any pesticides during their storage of grain. These may have enhanced the possibility of fungal grain infection under the storage conditions, as the result of this study indicated. For decades, the use of various synthetic pesticides has been the basis for the proper and long-term storage of cereals, primarily free of insects and mites but also fungi and their

mycotoxins and rodents. Post-harvest treatment of seeds and the prevailing environmental factors are key determinants of the impact that ton the quality of the seeds, including germinability. Proper management of the harvested grain is vital to overcome the problems that can be caused by biotic factors (microbial-based problems).

TABLE 4: Possible risk factors that contribute for fungal infection of wheat (*Triticum aestivum* L.) and faba bean (*Vicia faba* L.) under storage conditions

Risk Factors	Options	Sampling areas in Uraga district														
		HHM	X²	P value	HH	X²	P value	SK	X²	P value	RB	X²	P value	YB	X²	P value
DSIM	3	18.2	21.20	0.0001	9.0	26.20	0.0001	0	22.0	0.0001	27.3	1.6	0.4	18.2	40.9	0.0001
	6	27.3			45.5			0			36.4			63.4		
	> 12	54.5			45.5			100			36.4			18.2		
DSIY	1	0			9.0			0			0			18.2		
	2	45.5	50.70	0.0001	0	150.9	0.0001	9.0	150.9	0.0001	27.3	80.2	0.0001	27.3	21.3	0.0001
	3	54.5			91.0			91.0			72.7			54.5		
SM	Silos	18.2			27.3			27.3			0			9.0		
	Plastic sacks	54.5	21.20	0.0001	45.5	6.50	0.037	72.7	8.90	0.012	72.7	80.2	0.0001	63.7	45.8	0.0001
	Non-plastic sacks	27.3			27.3			0			27.3			27.3		
AFDC	Yes	27.3	1.70	0.19	54.5	0.10	0.800	18.2	1.80	0.2	36.4	0.7	0.4	63.6	0.7	0.4000
	No	72.7			45.5			82.0			63.6			36.4		
ECHT	Rainy	36.4			9.0			9.0			9.0			0		
	Dry	9.0	31.10	0.0001	45.5	26.20	0.0001	18.2	70.40	0.0001	27.3	34.2	0.0001	36.4	60.5	0.0001
	Cloud	54.5			45.5			72.7			54.5			63.6		
EMSP	Yes	45.5	0.10	0.77	63.6	0.70	0.400	82.0	2.90	0.1	54.5	0.1	0.8	63.6	0.7	0.4000
	No	54.5			36.4			18.0			45.5			36.4		
EFDA	Yes	45.5			27.3			72.7			63.6			72.7		
	No	54.5	0.10	0.77	72.7	1.70	0.200	27.3	1.70	0.2	36.4	0.7	0.4	27.3	1.7	0.2000
UOP	Yes	45.5	0.10	0.77	36.4	0.70	0.400	18.0	2.90	0.1	54.5	0.1	0.8	36.4	0.7	0.4000
	No	54.5			63.6			82.0			45.5			63.6		

HHM- Harsu Haro Muda, **HH**- Harsu Haro Hadama, **SK**- Suke, **RB**- Raro Boda, and **YB**-Yabitu.

DSIM- durations of storage in months, **DSIY**- durations of storage in years, **SM**- storing materials, **AFDC**- application of fungicides at field conditions during cultivation, **ECHT**- environmental climatic conditions at the harvesting time, **MMHC**- means of mowing the crop from the field during harvest collection, **EMSP**- existence of moisture in the storage places, **EFDFA**- the existence of fungal disease in the farming areas and **UOP**-Use of pesticide for storage.

TABLE 4 (continued)

Risk Factors	Options	Sampling areas in Uraga district																	
		HW	X²	P value	LT	X²	P value	RM	X²	P value	HHR	X²	P value	HRr	X²	P value	HS	X²	P value
DSIM	3	9.0	26.2	0.0001	27.3	6.6	0.04	36.4	1.6	0.4	18.2	21.3	0.0001	9.0	150.8	0.0001	0	80.2	0.0001
	6	45.5			27.3			27.3			27.3			0			27.3		
	> 12	45.5			45.5			36.4			54.5			91.0			72.7		
DSIY	1	9.0	26.2	0.0001	27.3	80.2	0.0001	27.3	21.3	0.0001	0	111.4	0.0001	36.4	11.5	0.003	0	11.4	0.0001
	2	45.5			0			18.2			18.2			18.2			18.2		
	3	45.5			72.7			54.5			82.0			45.5			82.0		
SM	Silos	54.5	50.7	0.0001	18.2	11.5	0.003	45.5	26.2	0.0001	23.3	85.4	0.0001	0	80.2	0.0001	72.7	80.2	0.0001
	Plastic sacks	45.5			36.4			9.0			0			27.3			27.3		
	Non-plastic sacks	0			45.5			45.5			72.7			72.7			0		
AFDC	Yes	63.6	0.69	0.407	36.4	0.7	0.41	27.3	1.7	0.2	18.0	2.9	0.000 1	9.0	3.8	0.1	27.3	1.7	0.200
	No	36.4			63.6			72.7			82.0			91.0			72.7		
ECHT	Rainy	54.5	31.1	0.0001	27.3	6.6	0.038	63.6	45.8	0.0001	0	1.13	0.0001	0	150.9	0.0001	9.0	70.4	0.0001
	Dry	9.0			45.5			9.0			45.5			9.0			18.2		
	Cloud	36.4			27.3			27.3			54.5			91.0			72.7		
EMSP	Yes	72.7	1.71	0.2	45.5	0.1	0.8	72.7	1.7	0.2	45.5	0.08	0.8	91.0	3.8	0.1	82.0	2.9	2.900
	No	27.3			54.5			27.3			54.5			9.0			18.0		
EFDFA	Yes	54.5	0.08	0.8	63.6	0.7	0.41	72.7	1.7	0.2	45.5	0.08	0.8	9.0	3.8	0.1	27.3	1.7	0.200
	No	45.5			36.4			27.3			54.5			91.0			72.7		
UOP	Yes	54.5	0.08	0.8	27.3	1.7	0.19	36.4	0.7	0.41	45.5	0.08	0.8	45.5	0.1	0.8	45.5	0.1	0.800
	No	45.5			72.7			63.6			54.5			54.5			54.5		

HW- Haro Wachu, **LT**- Lacho Torka, **RM**- Raro Midhidi, **HHR**- Haro Haru, **HRr**- Hada Raro, and **HS**- Harsu Sala.

DSIM- durations of storage in months, **DSIY**- durations of storage in years, **SM**- storing materials, **AFDC**- application of fungicides at field conditions during cultivation, **ECHT**- environmental climatic conditions at the harvesting time, **MMHC**- means of mowing the crop from the field during harvest collection, **EMSP**- existence of moisture in the storage places, **EFDFA**- the existence of fungal disease in the farming areas and **UOP**-Use of pesticide for storage.

4. Conclusion and Recommendations

The present study revealed that the genera of *Aspergillus* and *Fusarium* were the two common fungal genera that infect faba bean and wheat grains under storage in the study parameters of fungal infection of seed grains under storage. The presence of different genera of fungi associated with wheat and faba bean seed grain samples observed in this study could be an indicator for a serious health problem. Thus, the biology and management of major storage fungi associated with wheat and faba bean should be given due attention, and identification of the fungi should be done at the species level using molecular study, and training on fungal infection of grain under storage conditions and their management strategies should be given to the producers and farmers paying attention to post-harvest yield management.

area, with the first and second ranks in wheat and faba bean, respectively. As contributing factors, the length of storage and moisture can be considered as the two important

Ethical approval

Ethical approval was obtained from Madda Walabu University, and a support letter for seed sample collection was obtained from the Department of Biology.

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Availability of data and materials

The data used for this study will be available upon request from the corresponding author.

Authors' contributions

TB designed the study, performed all the laboratory work, collected the data, and wrote the first draft of the manuscript. ZF conducted the statistical analysis, interpreted the analysis results, read, edited, and rewrote the first draft of the manuscript written by TB.

Competing interest

Authors have declared that no competing interests exist.

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