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Quarantine Border Management of *Tilletia* Associated with Wheat Grain: Indonesia Perspective

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Abstract

The fungus *Tilletia* species is a pathogen in wheat (*Triticum aestivum*) that always contaminates wheat grain import. It has always been a major obstacle in wheat import trade to Indonesia, especially in managing wheat grain in the port of entry. The interception from 2014 to 2019 showed that three species *Tilletia indica*, *T. tritici*, and *T. leavis* belong to A1 Quarantine Pests were intercepted. However, border management by relevant agencies at the port of entry as well as proper management of flour industry by conducting reasonable standard procedures and processing plants, could minimize the risk of spreading *Tilletia*. Successful elimination of the fungus *Tilletia* through milling processing by temperatures above 56 ^{o}C might be killed *Tilletia*. Taking into consideration that *Tilletia* as A1 quarantine pests, however, it could be considered low risk to import wheat grain to Indonesia. The procedure for handling imported wheat grain from ships to silos must ensure that it is carried out properly and does not seep out of the port border area and burn all was filtered during flour processing.

Keyword – Inspection – Pre-shipment – Processing – Recommendation

Introduction

Wheat commodity is an important crop consumption of the global economy due to its role as a main Cerealia crop that provides daily calorie and protein needs for approximately 60% of the global population and also for livestock (Shehzad et al. 2012). Up to now, Indonesia is still categorized as one of the largest imported wheat grains in the world (Zuroaidah et al. 2012). The need for wheat grains in Indonesia cannot be avoided, both as a basic ingredient for making wheat flour for industrial purposes, food, as well as being used as household preparations (Padauleng et al. 2018). According to report from 2010 to 2019, wheat importation volume and value of Indonesia was 75.6 million tons and predicted to value as much as 21 billion USD (CBS 2021). The high import of wheat grains into Indonesia is not only due to the demand for food consumption from wheat and for animal feed, it is also caused by the fact that Indonesia is not a wheat-producing country.

Generally, imported wheat grains into Indonesia were from Australia, Canada, the Federation of Russia, the United States of America (USA), India, Ukraine, Bulgaria, Brazil, Argentina,

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Singapore, and Bulgaria (Padauleng et al. 2018, CBS 2021). The high import of wheat into the territory of Indonesia also allows the entry of quarantine pests that may be associated with imports of wheat grains into Indonesia. Long-distance transport of raw commodities of both domestic and international trade has grown as the key driver of pest and disease spread (Aukema et al. 2010, Bain et al. 2010, Warziniack & Thompson 2013).

Quarantine pests on wheat grain such as *Tilletia indica* (syn. *Neovossia indica*), *T. tritici* (syn. *T. caries*) and *T. laevis* (syn. *T. foetida*) shall be prevented from entering the territory of the Republic of Indonesia (IAQA 2020, MoA 2020). Karnal bunt is a rust pathogen (*Tilletia* spp.) that often associates with wheat grains between countries. The prevention, control and elimination of transboundary pests and diseases is more than a national public good. Because of cross-border spread, effective protection is only possible through a concerted and coordinated effort among neighbouring countries. The control efforts of individual countries may be continually frustrated by neighbouring countries not taking equivalent action. An international approach also allows better advantage to be taken of natural geographic barriers and broader biological and epidemiological patterns (Brennan et al. 1990). The most likely pathway of entry *Tilletia* spp. is via international trade of seed and grain of wheat or triticale that has been infected with or contaminated with *T. indica*. Wheat seed is grain which will be used to sow wheat crops and is thus of the highest risk of resulting in an outbreak of *T. indica* (Barton & Lacey 2021).

Previous studies on the detection of *Tilletia* spp. on imported wheat grain have been studied by Zuroaidah et al. (2012), Padauleng et al. (2018), and Handayani et al. (2018). While one publication dealing with the detection of weed seeds associated with wheat grain has been published by Wildaniyah & Handayani (2020). Current study of *Tilletia* spp. associated with imported wheat grain to Indonesia was reported by Musdalifah (2021). These study results showed that some species of *Tilletia* spp. have been detected. Therefore, even though Indonesia is not a wheat producer, on the other hand, it is well known that Indonesia as a rice-producing country. It must be watched out for where the main host of *Tilletia* spp. It is a wheat plant and several plants belonging to the Gramineae family, which of course the rice plant is also a plant from the Gramineae family. Wildaniyah & Hadayani (2020) reported that observations of weed seeds in imported wheat grain showed that weed seeds collected from Australia, Canada and Ukraine consisted of 13 families and 20 species. Based on the above point of view, to minimize the risk of potential spread of *Tilletia* spp. to crops that might be sensitive to pathogens, the role of quarantine management at the border is very important. The importation of wheat grains arriving at the port must be closely monitored. Silo management must be managed maximally with high procedures, to maintain leakage wheat grains, as well as a processing system for certain shredded wheat grains in fungus *Tilletia* spp. is not be able to grow. Thus, this study aims are to strengthen quarantine border management of imported wheat grains to prevent dispersal *Tilletia* spp. to susceptible host plants.

Risk Associated with Imported Wheat Grain

To address the issue, the question arises how transboundary pathogen *Tilletia* spp. associated with wheat grain with reference to Quarantine point of view in Indonesia may give a significant impact to agriculture. The use of qualitative and quantitative data to analyzes the importance of transboundary pathogen to be prevented based on national interests. The data were taken by collecting annual report of the Agricultural Quarantine Agency from 2014 to 2019, journal articles, or any related documents from related agencies at entry points, and visiting the Agency of Border Agriculture Quarantine (Table 1, Fig. 1). Concerning geographical conditions and host, it was reported that *Tilletia* spp have a wide spread pathogen (Fig. 1).

Used Detection methodology

Morphological identification

Morphological identification of the fungus *Tilletia* spp., on wheat grain using washing test methods were used. Sterile distilled water and centrifuge are used to obtain fungal spores that settle in Eppendorf tubes (ISPM 2014, Padauleng et al. 2018). Identification of the morphology of the fungus *Tilletia* spp., including shape of the teliospores, color, and size of the diameter of teliospores (EPPO 2013, ISPM 2014, Padauleng et al. 2018). Testing of wheat grain containing *Tilletia* spp. can be done through several methods such as ISTA consisting of the filtration and hemocytometer methods.

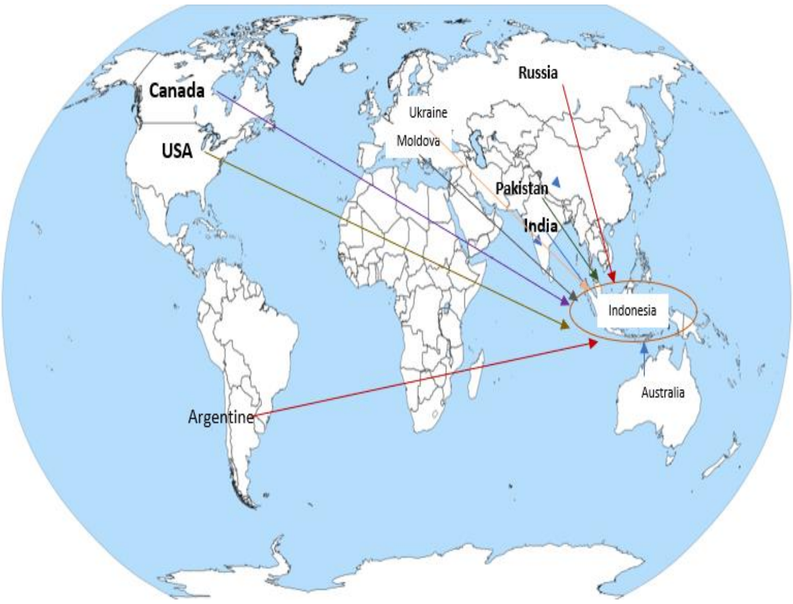


Fig. 1 – Map of the Importation of wheat grain to Indonesia.

Table 1 A1 Quarantine Pests of *Tilletia* spp. to Indonesia. MoA 2020.

No.	Rust Species of Wheat Grain	Host	Geographical Distribution
1	*Tilletia indica* (syn. *Neovossia indica*, (Mitra) Mundk., 1941); Karnal bunt of wheat, Indian bunt of wheat, partial bunt of wheat, new bunt	Wheat	America: Mexico, USA Asia: Afghanistan, India, Iran, Iraq, Nepal, Pakistan
2	*Tilletia tritici* (syn. *T. caries* (DC.) Tul., 1847; (Syn. *Caeoma sitophilum* (Ditmar) Link, 1825, *Fusisporium inosculans* Berk., 1847, *Lycoperdon tritici* Bjerk., 1775, *Tilletia caries* var. *agrostidis* Auersw., 1865, *Tilletia caries* var. *caries* (DC.) Tul. & C. Tul., 1847, *Tilletia sitophila* (Ditmar) J. Schröt., 1877,	*Agropyron* spp. (wheat grass), *Bromus* spp. (brome grasses), *Elymus* spp. (squirrel tail), *Festuca* spp. (fescue), *Hordeum* spp. (barley), *Poa* spp., *Secale sereale* (rye), *Lolium* spp., *Triticum* spp. (wheat)	**Africa**: Algeria, Egypt, Ethiopia, Lesotho, Libya, Morocco, South Africa, Tunisia, Uganda, Zimbabwe **America**: Argentine, Canada, Chile, Colombia, Ecuador, Guatemala, Mexico, Nicaragua, Peru, USA, Uruguay, Venezuela **Asia**: Afghanistan, Armenia, Azerbaijan, Bhutan, China, India, Iran, Iraq, Israel, Japan, Jordan, Kazakhstan,
3	*Tilletia laevis* J.G.Kuhn, 1873; (syn. *T. foetida*, Wallr, Liro, 1920); *Erysiphe foetida* Wallr., 1833, *Tilletia foetems* (Berk. & M.A Curtis) Trel., 1884; *Tilletia tritici* var. *laevis* (J.G. Kühn) Kawchuk, 1988, *Ustilago foetens*	*Secale cereale* (rye), and *Triticum aestivum* (wheat)	**Africa**: Algeria, Angola, Egypt, Ethiopia, Kenya, Lesotho, Libya, Morocco, Somalia, South Africa, Tunisia, Zimbabwe **America**: Argentine, Canada, Chile, Colombia, Mexico, Peru, USA, Uruguay,

Table 1 Continued.

No.	Rust Species of Wheat Grain	Host	Geographical Distribution
	Berk. & M.A Curtis, 1874).		Venezuela. **Asia**: Afghanistan, Armenia, Azerbaijan, China, DPR Korea (North Korea), India, Iran, Iraq, Israel, Japan, Kazakhstan, Lebanon, Mongolia, Nepal, Pakistan, Republic of Korea (South Korea), Saudi Arabia, Syria, Turkey, Turkmenistan, Uzbekistan **Europe**: (all EU member states) **Oceania**: Australia, New Zealand
4	*Tilletia controversa* J. G. Kühn, 1874; (Syn. *Tilletia aegilopsidis* Golovin, 1952, *Tilletia brevifaciens* G. W. Fisch., 1952, *Tilletia controversa* var. *controversa* J. G. Kühn, 1874, *Tilletia nanifica* F. Wagner ex Săvul., 1956, *Tilletia tritici* var. *controversa* (J. G. Kühn) Kawchuk, 1988, *Tilletia tritici* subsp. Nanifica F. Wagner, 1950, *Tilletia tritici-nanifica* F. Wagner, 1950).	*Agropyron* (wheatgrass), *Elymus* (wildrye), *Hordeum vulgare* (barley) (grasses), *Secale cereal*e (rye), *Triticum aestivum* (wheat)	**Africa**: Algeria, Libya, Tunisia America: Canada, USA Asia: Afghanistan, Georgia, Iran, Iraq, Japan, Kazakhstan, Kyrgyzstan, Syria, Tajikistan, Turkey, Turkmenistan, Uzbekistan **Europe**: Albania, Armenia, Austria, Bulgaria, Croatia, Czech Republic, Denmark, Greece, Hungary, Italy, Latvia, Luxemburg, Moldova, Montenegro, Poland, Romania, Russian Federation, Slovakia, Slovenia, Sweden, Switzerland, Ukraine
5	*Tilletia caries* (DC.) Tul., 1847; (Syn. *Caeoma sitophilum* (Ditmar) Link, 1825, *Fusisporium inosculans* Berk., 1847, *Lycoperdon tritici* Bjerk., 1775, *Tilletia caries* var. *agrostidis* Auersw., 1865, *Tilletia caries* var. *caries* (DC.) Tul. & C. Tul., 1847, *Tilletia sitophila* (Ditmar) J. Schröt., 1877, *Tilletia tritici* (Bjerk.) G. Winter, 1874, *Tilletia tritici* f. *tritici* (Bjerk.) G. Winter, 1874, *Tilletia tritici* var. *tritici* (Bjerk.) G. Winter, 1874, *Tilletia tritici* subsp. *tritici* (Bjerk.) G. Winter, 1874, *Uredo caries* DC., 1815; *Uredo sitophila* Ditmar,	*Agropyron* spp. (wheat grass), *Bromus* spp. (brome grasses), *Elymus* spp. (squirrel tail), *Festuca* spp. (fescue), *Hordeum* spp. (barley), *Poa* spp., *Secale sereale* (rye), *Lolium* spp., *Triticum* spp. (wheat)	**Africa**: Algeria, Egypt, Ethiopia, Lesotho, Libya, Morocco, South Africa, Tunisia, Uganda, Zimbabwe **America**: Argentina, Canada, Chile, Colombia, Ecuador, Guatemala, Mexico, Nicaragua, Peru, USA, Uruguay, Venezuela **Asia**: Afghanistan, Armenia, Azerbaijan, Bhutan, China, India, Iran, Iraq, Israel, Japan, Jordan, Kazakhstan, Kyrgyzstan, Lebanon, Mongolia, Pakistan, Republic of Korea (South Korea), Saudi Arabia, Syria, Taiwan, Turkey, Uzbekistan. **Europe:** (all EU member states).

Biomolecular detection

The detection of *Tilletia* spp. which infects wheat germ was carried out using a PCR technique using specific oligonucleotide primers to amplify the DNA extraction of the fungus *Tilletia* spp. (Padauleng et al. 2018). Meanwhile, Mulholland & McEwan (2000) reported that an effective method for testing *Tilletia* spp. using PCR consists of DNA Extraction, Competitive PCR Quantification, and Real time PCR (Killermann et al. 2008). While, testing protocols of *T. indica* and molecular methods consist of PCR and ELISA were used (EPPO 2013).

Fungus associated with imported wheat grain

A total of 33 frequency of intercepted *Tilletia* spp. during 2014 to 2019 consist of three A1 Quarantine pests such as *T. indica*, *T. tritici*, and *T. leavis* (Table 2). *T.trici* was found to contaminate wheat grains every year. *T. indica* was intercepted from imported wheat grain from India and Pakistan. Meanwhile, *T. tritici* and *T. laevis* were intercepted from imported wheat from Moldova, Ukraine, Argentine, and USA. Species *T. indica* and *T. titici* were found from Pakistan. The highest frequency of intercepted was found in the Species *T. tritici* with a total of 16 times, and is always intercepted every year (Table 2).

Table 2 Frequency of *Tilletia* spp. intercepted from imported wheat grain to Indonesia.

No.	Species	Country of Origin	Frequency of Intercepted (times)						Total Freq.
			2014	2015	2016	2017	2018	2019	
1	*Tilletia indica*	India, Pakistan	1	-	3	-	2	1	6
2	*Tilletia tritici*	Moldova, Ukraine, Russia, USA, Argentine, Pakistan, Australia	2	1	4	1	2	6	16
3	*Tilletia leavis*	Ukraine, Argentine, India, USA, Moldova, Canada	1	2	2	-	5	-	10
Total Frequencies			4	3	9	1	9	7	33

The study reported by Zuroaidah et al. (2012) showed that species *T. laevis* and *T. tritici* in wheat samples from Russia and *T.indica* from India were commonly intercepted. Furthermore, species *T. tritici* and *T. laevis* were also found in imported wheat grain from Ukraine and species *T. indica* was also detected in imported wheat grain from India (Padauleng et al. 2018). Musdalifah (2021) reported that wheat grain from the United States was infected with *T. tritici* and *T. indica*. Wheat grains from Moldova were infected with the fungus *T. laevis*. The presence of harmful non-native organisms causes damages to economically valuable host resources and negatively affects economically important crops (Warziniack et al. 2011). Fungus *Tilletia* spp. are also frequently intercepted in imported wheat grain to China (Zhang et al. 2011).

The unfavorable condition of this pathogen was difficult to survive because none of main host such wheat crops, when escaping to the suitable environment. This was in line with Smilanick et al. (1989) highlighted that effect of environmental conditions on its survival and growth of *Tilletia indica* have been determined under controlled conditions. Indonesia has long established trade link in wheat grain with countries where Karnal bunt existed, including India and Pakistan. For Indonesia to protect its own territory, measures need to be implemented to ensure that the risk is acceptably low. Stansbury & Kirdy (2002) mentioned that all movement of wheat grain into Australia, a measure put in place and shall be based on relevant international standards, guideline, and recommendations.

Table 2 showed that *T. tritici* was the highest frequency of detection on wheat grain from Moldova, Ukraine, Russia, Argentine, Pakistan, USA, and Australia. This was inline with this fact, although it is possible that entry of small number of spores could results in establishment of the pathogen (Murray et al. 1998).

Enforcement for Quarantine Border Control Management

Border institutions including Immigration, Customs, Quarantine, and Security Agencies in border checkpoint areas are border guards who facilitate trade, help suppress and prevent crimes that are not detected, and penetrate the country through an integrated screening process (Boriboonrat 2013). The problems cannot be handled by one institution alone, and the institutions involved have not been able to implement effective border management, so several border regulations and practices overlap in their application (Setiawan et al. 2020). Furthermore, Rusdiyanta (2017) highlighted that border guards also face complex problems such as human trafficking, people smuggling, transnational crime organizations, illegal migrants, and which require collaboration between agencies involved at border management to ensure that Indonesia obtain positive results from globalization through facilitating the movement of goods and people without ignoring the security aspects and preventing the entry of pests and diseases into the territory of Indonesia.

The movement of planting materials, trade and travellers can be vehicles for long distance transmission of plant diseases, such as *Tilletia* spp. on wheat is airborne and can easily be spread by the wind across borders. In this case, to ensure that border management runs optimally, it requires a win-win collaboration among institutions involved in border management Agency such as Customs, Immigration, Quarantine, and Security, to ensure Indonesia could improve prevention of the entry and spread of quarantine pests. On the other hand, it could facilitate the movement of goods and people. Those institutions involved must implemented effectively border control management (Setiawan et al. 2020).

Although border control is carried out by various institutions that have different tasks and functions, they have the same goal of protecting the sovereignty of a country, so border control must run effectively. Implementing border control requires cooperation and coordination with relevant agencies such as the Ministry of Health, Agriculture, Maritime Affairs and Fisheries, Home Affairs, Police, and Security (Rusdiyanta 2017). Integrating different levels of authority requires an approach with the same substantive objective so that the implementation of border control on imported wheat inspection activities can be carried out properly at the Port of entry.

Control border management approach can improve the efficiency of administrative procedures and reduce transaction costs and delays in transporting goods and people at the border (Rusdiyanta 2017). McLinden (2012) highlighted that control border management can increase country's competitiveness and exports as well as reduce the cost of international trade. In addition, increasing border cooperation, it could be reduced the occurrence of illegal activities (EC 2009). The existence of control border management is useful for reducing differences in the implementation of border policies both from the perspective of resources and economies of scale (Aniszewski 2009). Border management can increase transparency and reduce opportunities for fraud (Mclinden 2012).

Encourage Pre-shipment Quarantine Inspection

To maximize efforts to prevent contamination of *Tilletia* spp in imported wheat grain, the importing country may encourage a pre-shipment quarantine inspection in the exporting country before dispatching to the destination country. Both parties may enhance mutually technical cooperation for the operation of wheat trade for both parties.

Proper Silo Management to kill Teliospore of *Tilletia* spp.

To ensure the screening process of wheat industry, wheat is filtered through a screen separation machine that functions to separate wheat from other objects larger than wheat such as wheat stalks or other organic objects, objects that are lighter than wheat will be sucked in by the aspiration machine. After going through aspiration, the wheat is stored in the raw wheat bin. The wheat to be milled is weighed first (raw wheat weighing), then metal objects are caught by Ferrous Metal Trapping II, the wheat is then filtered again separated from stones and others (combi cleaning).

Wheat is separated based on its color by Indent Separation, lice eggs attached to the wheat husk will be destroyed by the Entoleter machine (insect destroying), the wheat grain will be brushed so that the wheat will be separated from the outer shell (1st scouring), the rubbing results will be separated by the machine aspiration, the wheat will be sprayed by water to make it easier to grind (1st tempering), after being sprayed with water, the wheat is left overnight for 24 hours in the tempering bin (1st tempering bin), wheat that has not received sufficient moisture will be resprayed with water (2nd tempering). Then it will be stored back into the tempering bin (2nd tempering bin), the wheat that has been sprayed with water is then rubbed again (2nd scouring), the dregs from rubbing will be separated through the machine (Fig. 2). Wheat grain treatment to eliminate a pathogen of *Tilletia* spp. associated with wheat grain (a) wheat intake process; (b) wheat transfer, cleaning & tempering process; (c) milling process; and (d) finished product handling process (Zuroaidah et al. 2012).

After the pollard is formed, it is ensured that free from contamination by all living organisms including the fungus *Tilletia* spp. This is due to the process from the formation of pollard to the process of pellets, the temperature used is above 56 ^{o}C, causing the death of teliospores of *Tilletia* spp. This fact is line with the statement of Gultyqeva (2009) stated that the relative humidity of teliospore germination is between 40-60% and a temperature that supports teliospore survival of 30-50 ^{o}C. From this point of views, contaminated wheat grain with *Tilletia* spp could be eliminated by given a heating treatment at temperatures above 56 ^{o}C (Fig. 2).

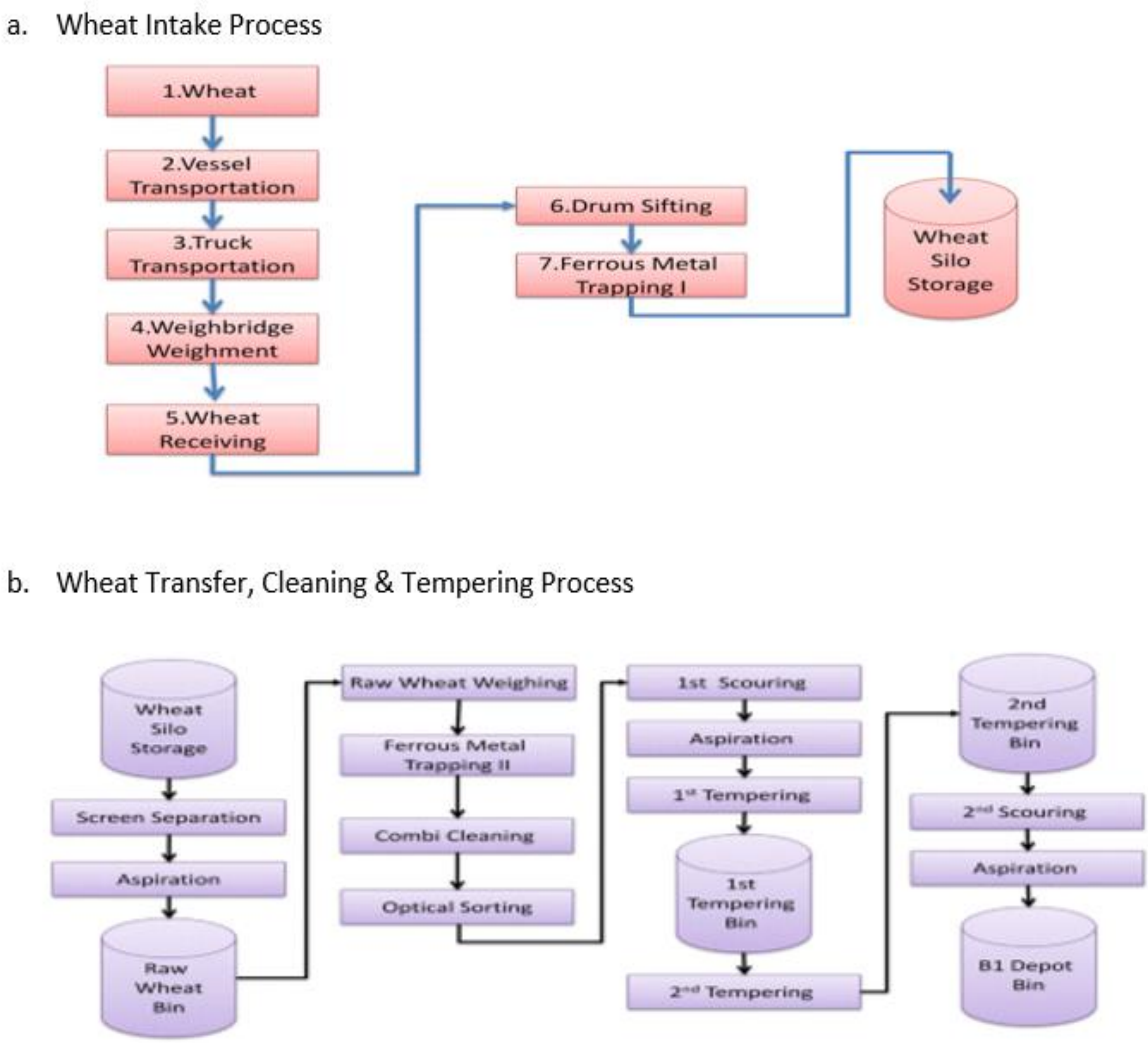
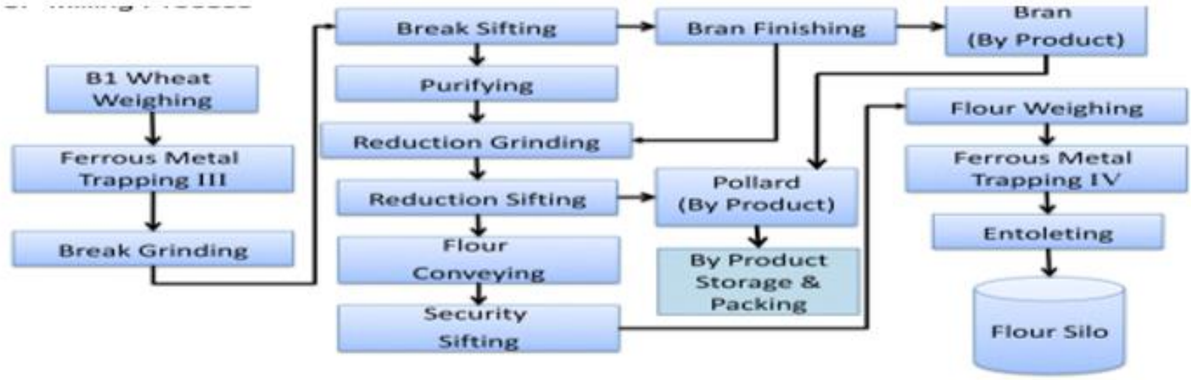


Fig. 2 – Flowchart of proper silo management for killing *Tilletia* spp. on wheat grain (a, b, c, and d) (Zoroaidah et al. 2012).

c. Milling Process



d. Finished Product Handling Process

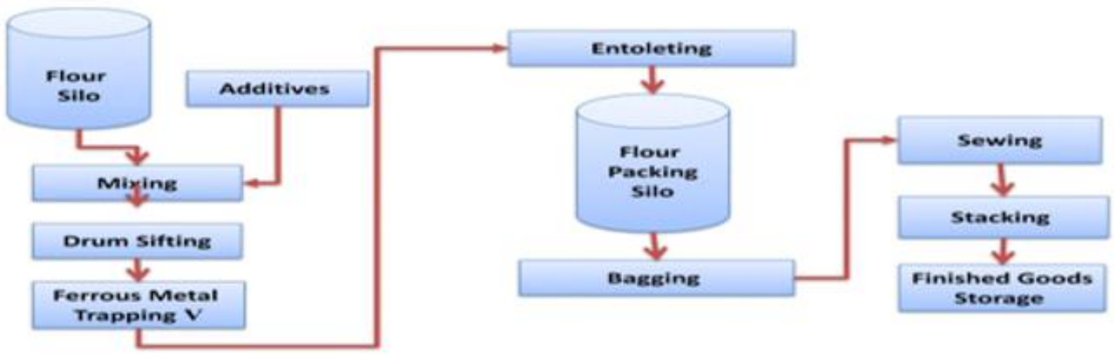


Fig. 2 – Continued.

Conclusion

To conduct proper border quarantine management at the port of entry, The Plant Quarantine Agency may establish a joint procedure with the wheat flour industry regarding management of wheat imports by applying the proper plant biosecurity.

Successful elimination of the fungus *Tilletia* spp. through milling processing by temperature above 56 ^{o}C which may kill teliospora *Tilletia*, it was recommended that even though *Tilletia* spp belongs to A1 Quarantine Pests, they could be considered as low risk of wheat grain. The procedure for handling imported wheat grain from ships to silos must ensure that it is carried out properly, does not seep out of the port border area, and burns all waste filtered during flour processing.

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