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FLOW OF QUARANTINE REQUIREMENTS AND CHARACTERIZATION OF *Tilletia sp.* ON WHEAT IN DETERMINING IMPORT APPROVAL

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Abstract
The high volume of wheat imports in 2022, which reached 9.35 million tons, increases the risk of entry of the quarantine plant pest organism Tilletia sp., classified as Group 1 Category A1. These groups and categories cannot be treated and do not yet exist in Indonesia. Therefore, monitoring the fungus Tilletia sp. must be severe and handled appropriately. This activity aims to understand the flow of quarantine requirements and characterization of Tilletia sp. as a determinant of import approval at the Animal, Fish, and Plant Quarantine Center. The flow of quarantine requirements is implemented when the importer prepares the application file online, Phytosanitary Certificate, Invoice, Bill of Lading, Shipping Instruction, Prior Notice, Certificate of Analysis, Health Certificate, and Application for Import. Wheat commodities, if infected with Tilletia sp., will be detained, and a detention warrant will be issued; if treatment is not given, a destruction order and an extermination report will be issued. Characterization of Tilletia sp. was determined based on the frequency of washing test results. Wheat samples with Tilletia sp. >10 times must be treated with heat treatment to kill the development of spores.

Keywords*: Wheat Import, Quarantine, Characterization, Washing Test, Tilletia sp.*

INTRODUCTION

The level of wheat consumption in Indonesia is not balanced with domestic production, so that dependence on wheat imports cannot be avoided (Cipta & Asmara, 2023; Nugroho & Faridatussalam, 2023). The high consumption of wheat derivative products as raw materials for making household and industrial food needs, such as instant noodles, bread, instant cereals, and biscuits, shows a shift in the high level of wheat consumption in Indonesia (Putri & Karmini, 2023). To meet its current wheat needs, Indonesia imports wheat from various countries such as Australia, Ukraine, Canada, Argentina, the United States, India, Bulgaria, Brazil, Rep. Moldova, Fed. Russia, etc (Table 1) (Statistic Indonesia, 2023).

Table 1. Indonesia wheat import data 2017-2022

Country of Origin	2017	2018	2019	2020	2021	2022
	Net Weight (million tonnes)					
Australia	5.098,2	2.419,7	891,4	830,8	4.626,4	4.192,0
Ukraina	1.822,2	2.411,0	2.984,2	2.953,2	2.833,8	166,8
Kanada	1.686,1	1.973,7	2.439,3	2.336,6	1.919,1	1.322,4
Argentina	152,3	678,0	1.952,5	2.635,5	606,8	1.469,7
Amerika	1.150,1	904,2	1.256,2	1.277,0	447,9	392,4
India	0,0	0,0	0,0	0,0	318,5	908,1
Bulgaria	0,0	0,5	170,9	129,4	227,6	167,5
Brasil	64,4	0,0	266,5	0,0	123,0	641,6
Rep. Moldova	57,5	53,7	111,9	55,7	65,9	30,4
Fed. Rusia	1.189,3	1.228,1	515,9	68,8	3,0	0,0
Lainnya	5,6	414,7	75,5	0,0	0,0	59,5
Total	11.225,6	10.083,4	10.664,2	10.287,1	11.172,0	9.350,4

Source: (Statistic Indonesia, 2023)

The wheat flour industry in Indonesia is expected to continue to grow due to the high amount of wheat imports in 2022, which reached 9.35 million tons (World-grain, 2023). Wheat is an important commodity for which no alternative substitute has been found. High wheat imports carry

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the risk of the possible introduction of quarantine plant pests *Tilletia* sp., which are known to cause bunt disease. Bunt disease reduces the quality of imported wheat, characterized by blackish brown seeds, stunted seeds, and malformations, so that the processed quality is also poor (Gao et al., 2015; Wiyono et al., 2022; Yuliawati, 2021). Serious attention is required regarding the potential introduction of Tilletia sp., considering that imported wheat is widely utilized in food-processing industries. Regulation of the Minister of Agriculture Number 25 of 2020 states that *Tilletia* sp. is included in quarantine plant pests group 1 category A1, which cannot be treated and has not been detected in Indonesia (Ministry of Agriculture of the Republic of Indonesia, 2020). Therefore, supervision of the *Tilletia* sp. fungus must be a serious concern and needs to be handled properly.

The results of the characterization of wheat seeds proven to be infected with *Tilletia* sp. must be tested in the laboratory to obtain accurate data as a basis for determining the decision to import commodities from various countries. This supervision is carried out by the Animal, Fish, and Plant Quarantine Center with the specifications of quarantine action tasks according to Government Regulation Number 29 of 2023, including inspection, isolation, observation, treatment, detention, rejection, destruction, and release (Government of Indonesia, 2023). Importers must understand the document requirements for transaction purposes so that imported wheat commodities are not detained, rejected, or destroyed by quarantine authorities. The Animal, Fish, and Plant Quarantine Center is tasked with providing guarantees for the realization of food security in Indonesia through a fast and accurate supervision system.

The urgency of this activity is to prevent the entry of imported wheat commodities infected with *Tilletia* sp. so that the processed products are of high quality. The formulation of the problem of this activity is how the flow of wheat imports and the characterization of *Tilletia* sp. in wheat as a determinant of commodities being detained or released. The purpose of this activity is to understand the flow of quarantine requirements and the characterization of *Tilletia* sp. in wheat as a determinant of import approval.

RESEARCH METHODS

Research Type and Design

The research was designed as a descriptive observational study, focusing on the characterization of quarantine plant pest organisms found in imported wheat commodities. Documentation was carried out during the internship. The documents collected and summarized include data on wheat import requirements and the results of the characterization of *Tilletia* sp. as the basis for determining the entry of wheat import commodities.

Location and Time of Research

The activity in the research of checking the quarantine requirements flow and characterization of quarantine pests for imported wheat commodities was carried out at the Semarang Container Terminal. Sample testing was carried out at the Animal, Fish, and Plant Quarantine Center, Central Java, mycology laboratory. The activity implementation period is 4 months from July 17-November 17, 2023.

Activity Procedure

Receiving Documents and Checking Data for Wheat Import Requirements

Wheat commodity importers fulfill several document requirements for quarantine. The document requirements are presented in Table 2.

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Table 2. Wheat import requirements data

Submission Data	Description
Online application	Submission of the quarantine process from importer
Phytosanitary Certificate from Country of Origin	Document guaranteeing that the exported products are free from insects, pests, soil contamination, and disease
Invoice	Proof of transaction, made by the exporter for importer
Bill of Lading	Proof of delivery of goods/receipt made by the shipping company for the exporter
Shipping Instruction	Documents from exporters are forwarded to shipping companies to book the commodity transport vessels
Prior Notice	Certificate of fresh food of plant origin
Certificate of Analysis	Results of chemical compound analysis of products exported from the country of **registration**
Health Certificate	Results of chemical compound analysis of products exported from **non-registered** countries
Application for Import	Import application submitted to the quarantine office

Wheat Sampling

Wheat sampling was carried out in the hold of a ship docked at Tanjung Emas Port, Semarang, PT. Sriboga area (Figure 1). Wheat samples were taken 4 times during the period 17 July-17 November 2023. Wheat commodities imported from Ukraine were taken 2 times, Bulgaria 1 time, and Canada 1 time.

Characterization Test of Tilletia sp. Fungus

Characterization of *Tilletia* sp. was carried out using a washing test (Figure 2). The specifications of the tools and materials for characterization are described in Tables 3 and 4. Wheat samples were sorted ± 400 grains from seeds that appeared to have symptoms of a blackish-brown spore mass and then placed in an Erlenmeyer flask (Figure 2a).

A 0.01% tween 20 solution was added to the test sample to a volume of 100 ml (Figure 2b). The purpose of using a 0.01% Tween 20 solution was to extract teliospores from the seed surface (Abshire, 2023). The sample was placed in a shaker for 5 minutes (Figure 2c), then the solution was poured into a 15 ml tube and centrifuged for 10 minutes at 3000 rpm to obtain a spore sediment of the fungus (Figure 2d). The supernatant was discarded, and 2 ml of lactophenol was added to the pellet (coloring is optional). A small amount of pellet was taken using a dropper and placed on a glass object containing sheer medium and covered with a glass deck. The preparation was observed with a compound microscope, documented, and characterized with the help of NIS D-Element software (Figure 2e).

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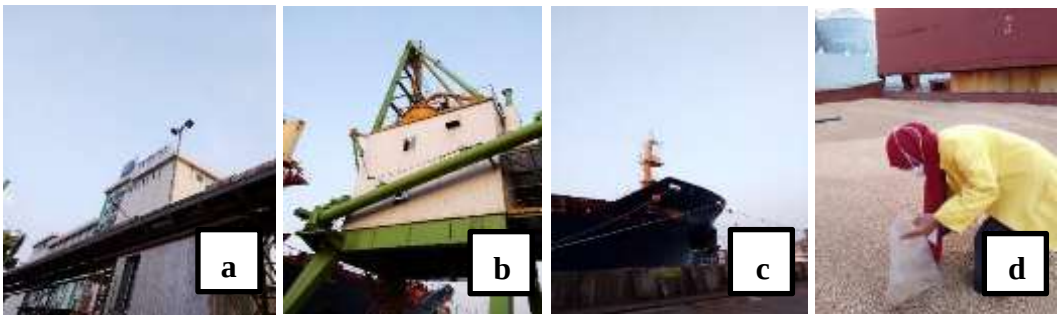

Figure 1. Sampling of wheat at Semarang Container Terminal; (a) PT. Sriboga as the importer; (b) storage room for imported wheat at PT. Sriboga; (c) ship carrying imported wheat; (d) sampling of wheat from the ship.

Figure 2. Characterization test of *Tilletia* sp. fungus from imported wheat samples. (a) sorting of symptomatic wheat seeds; (b) testing with washing test; (c) sample shaker to separate *Tilletia* sp. spores from wheat seeds; (d) sample centrifugation to obtain spore pellets; (e) observation of spores for characterization of *Tilletia* sp. fungus.

Data Analysis Techniques

The characterization of the wheat quarantine pest was carried out through morphological observations and digital micrometric results to measure the diameter of *Tilletia* sp. teliospores. Ministry of Agriculture of the Republic of Indonesia 25 of 2020 states that the types of wheat commodity quarantine pest are the fungi *Tilletia caries/T. tritici, T. laevis, T.controversa*, and *T. indica* (Ministry of Agriculture of the Republic of Indonesia, 2020). The frequency of fungal findings states that wheat is infected with *Tilletia* sp. if more than 10x spores are found.

RESULTS AND DISCUSSION

Characterization Flow of wheat import requirements

Wheat importers begin their import activities according to the flow of wheat import application requirements by filling out the quarantine inspection application form on page PPK Online (pertanian.go.id). The online requirements are met, and then the original documents are submitted to the Animal, Fish, and Plant Quarantine Center, Central Java. The operator follows up on the IQ-FAST page process at the Customs Processing Center for the Indonesian National Single Window System (PPK SSM-INSW) to create a PPK data accuracy analysis assignment letter as supporting document 1 (DP1). The operator then issues DP2 as a report on the results of the administrative inspection. The operator then issues a Letter of Approval for the

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Implementation of Plant Quarantine Actions Safety Supervision (KT-2) so that samples of imported wheat commodities can be taken from the ship's hold docked at the entry point for health inspection.

The health inspection is complete, the operator then makes a report on the results of the health inspection (DP-5), and the importer completes the payment for the commodity inspection process. If the commodity is decided to be temporarily detained, a detention letter (KT-8) will be issued. The commodity is destroyed when the importer and exporter do not give a decision to "give treatment," so that a destruction order (DP-10) and a destruction report (KT-14) are issued. If the commodity is not infected with *Tilletia* sp., a release certificate (KT-9) can be issued immediately.

Characterization results of *Tilletia* sp. fungi on imported wheat

Wheat seed characterization was carried out in the mycology laboratory of the Animal, Fish, and Plant Quarantine Center, Central Java, using a washing test as the basis for deciding the treatment that should be given to imported commodities. Heat treatment was used to suppress 56.7–61.7% of *Tilletia* sp. teliospora germination without the need to reduce wheat protein levels (Handayani et al., 2018). Wheat is declared infected with quarantine pest if the frequency of *Tilletia* sp. findings is >10 times. The results of *Tilletia* sp. identification are determined based on the Animal, Fish and Plant Quarantine Center, Central Java fungal identification guidebook, and the research results of Oepp & Bulletin (2018). The specifications are as follows.

- *Tilletia caries*/*T. tritici*: has round teliospores, 14-24 µm in diameter, with relatively short reticulation.
- *T. laevis*: has ellipsoidal to round teliospores, 14-22 µm in diameter. The cells have smooth exospores and a thin mucoid sheath.
- *T. controversa*: round, 16-25 µm in diameter. The cells have net exospores and are highly reticulated with a hyaline mucoid sheath.
- *T. indica*: has teliospores with a diameter of 22-49 µm with pale orange-brown to dark reddish brown teliospores; mature spores are dark black. The outer surface is composed of densely echinulate & finely cerebriform spines. The characterization results of imported wheat from 4 test samples are presented in Table 5.

Table 3. Characterization results of the fungus *Tilletia* sp.

Country of origin	Frequency of Findings				Final Decision
	T. caries/ T. tritici	***T. laevis***	***T. controversa***	***T. indica***	
Ukraine sample 1	6	1	3	1	*Tilletia* sp. findings >10x, Commodities need to be given heat treatment
Ukraine sample 2	6	3	5	2	Commodities need to be given heat treatment
Bulgaria	0	0	0	0	Commodities are released
Kanada	0	0	0	0	Commodities are released

Source: Primary Data (2023)

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Quarantine pest findings in the Ukraine wheat sample 1 were *Tilletia caries*/*T. tritici* spores with round teliospores, 14-24 µm in diameter, with relatively short reticulation. The spore diameters of findings 1 (16.01 µm and 18.65 µm), findings 2 (16.59 µm and 18.45 µm), findings 3 (15.07 µm and 15.16 µm), findings 4 (15.15 µm and 19.65 µm), findings 5 (18.27 µm and 19.32 µm), and findings 6 (14.81 µm and 15.70 µm) (Figure 3a -f). *T. laevis* fungus was found once in the sample without lactophenol staining with round light brown teliospores, smooth exospores equipped with a thin mucoid sheath with a diameter of 19.05 µm and 20.94 µm in the range of 14-22 µm (Figure 3g). The diameter of *T. controversa* 16-25 µm with net-shaped and highly reticulated exospores was found 3 times. The diameters of findings 1-3 were respectively 17.43 µm & 19.10 µm; 16.81 µm & 18.23 µm; and 18.99 µm & 19.39 µm (Figure 3h-j). *T. indica* with teliospores with a diameter of 22-49 µm equipped with exospores consisting of adjacent and smooth spines was found 1 time with a diameter of 24.50 µm & 27.50 µm (Figure 3k).

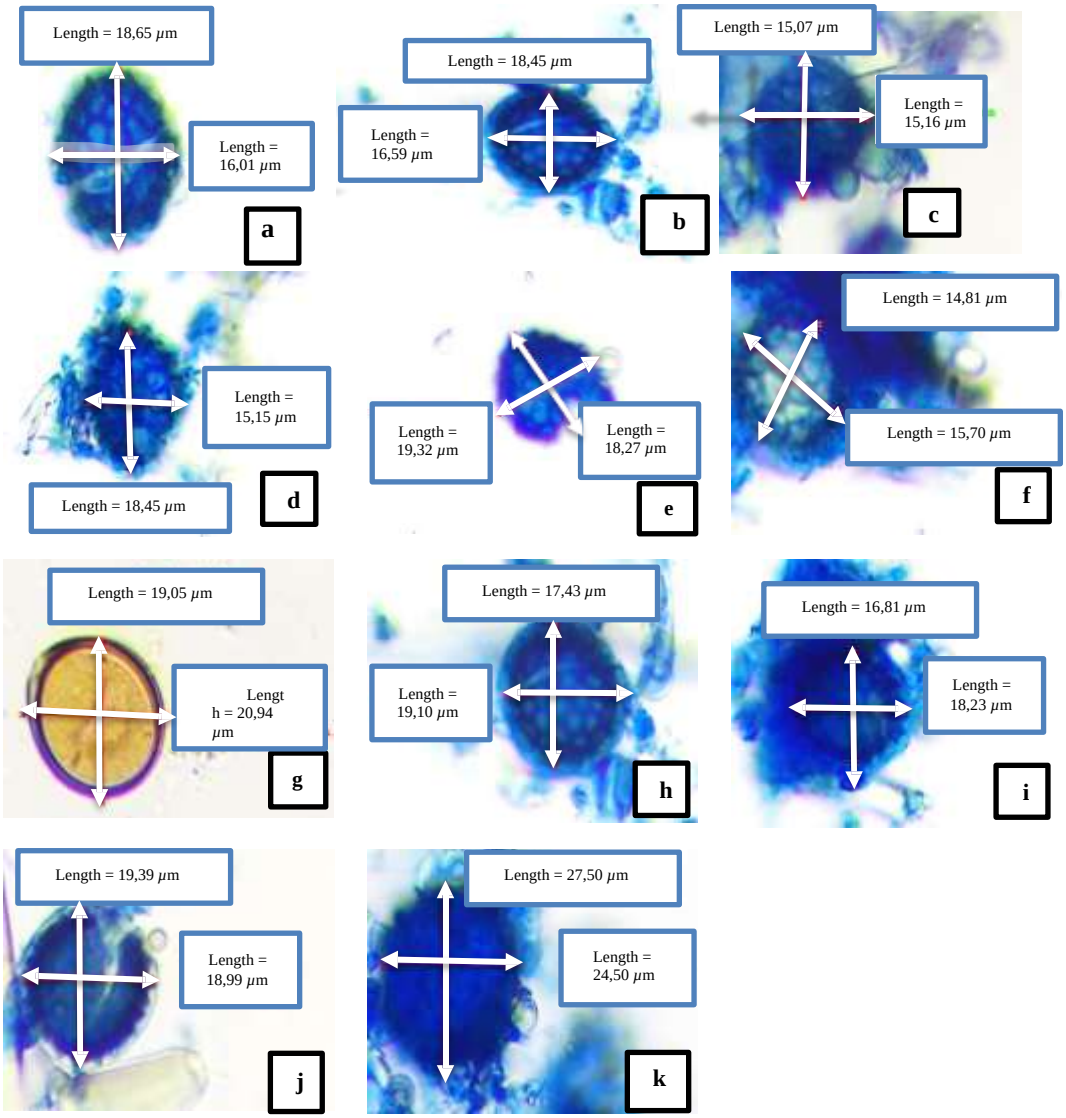

Figure 3. Findings of *Tilletia* sp. magnification 40x in sample 1, imported wheat from Ukraine. (a-f) spores of *T. caries/T. tritici*; (g) spores of *T. laevis*; (h-j) spores of *T. controversa*; (k) spores of *T. indica*.

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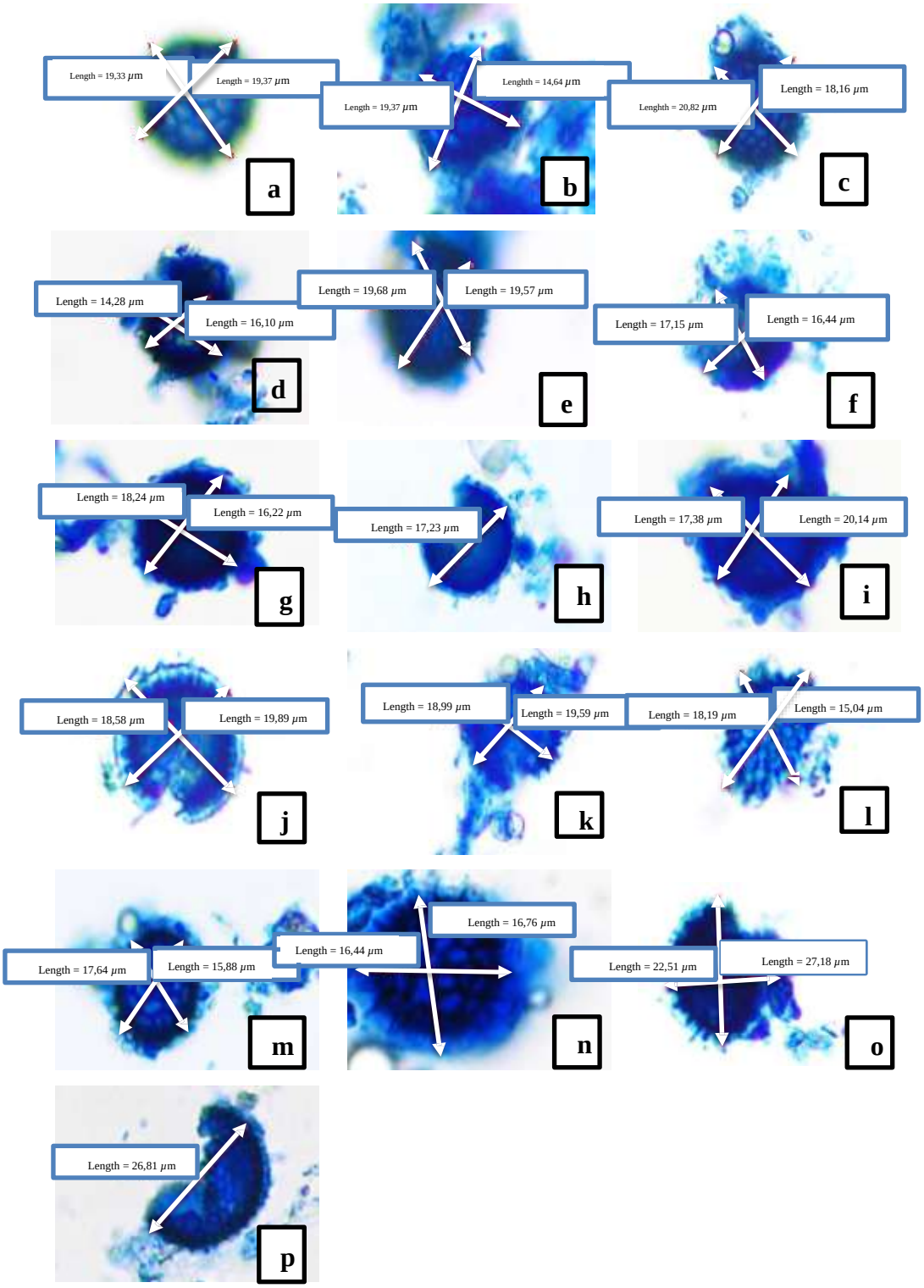

Figure 4. Findings of *Tilletia* sp. magnification 40x in sample 2, imported wheat from Ukraine. (a-f) *Tilletia caries* / *T. tritici* spores; (g-i) *T. laevis* spores; (j-n) *T. controversa* spores; (o,p) *T. indica* spores.

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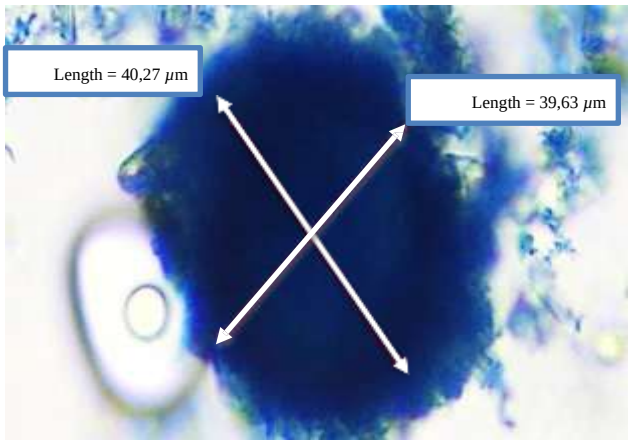

Figure 5. Suspect *T.indica* magnification 40x. Suspect identified diameter 39.63 µm & 40.27 µm (in the range of 22-49 µm), but the exospores are not composed of adjacent and smooth spines. Possible lysis of the seed part due to the use of the Tween 20 solution.

CONCLUSIONS AND SUGGESTIONS

The quarantine requirements that must be fulfilled by the importer are online application files, Phytosanitary Certificate, Invoice, Bill of Lading, Shipping Instruction, Prior Notice, Certificate of Analysis, Health Certificate, and Application for Import. Complete data is then made into a DP1 assignment letter and a DP2 administrative inspection report. The plant quarantine action approval letter (KT-2) and health examination results (DP-5) are made as the basis for billing payment, and then a release certificate (KT-12) is issued. If the wheat commodity is detained, a detention letter (KT-8) is made, and if it is not treated, a destruction order (DP-10) is made, complete with a destruction report (KT-14). The results of the *Tilletia* sp. characterization are determined based on the frequency of washing test findings. The test results of wheat samples 1 and 2 from Ukraine found *Tilletia* sp. >10 times, so that the wheat commodity must be given heat treatment to kill the development of spores. Wheat samples from Bulgaria and Canada did not contain *Tilletia* sp., so they can be released immediately.

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