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REVIEW ARTICLE

Pest Risk Assessment of Wheat Grains Import from Pakistan Contaminated with the Fungal *Tilletia indica* as Pest Quarantine A1: (A Novel of Academic Investigation)

Nur Amin[1*]
[1]Department of Plant Protection, Faculty of Agriculture, Hasanuddin University, Makassar 90245, South Sulawesi–Indonesia
* Corresponding author e-mail: nuramin_62@yahoo.com

ABSTRACT
The aims of the academic investigation are to give information about the necessity of holistic approaches in doing pest risk assessment, to collaborate among stakeholders in order to prevent dispersal of pest and disease quarantine in new areas, and to recommend strongly stakeholders related to case of wheat grains import in South Sulawesi. According to the Indonesian Government Regulation No.14 of 2002 about ***"plant quarantine"*** *that every carrier media, whose contamination with pest categories A1, has to be assessed through procedure of pest risk treatment and be destroyed indeed. In 2011, in Makassar South Sulawesi, the importer, Letifindo Company, imported wheat grains from Pakistan to Makassar South Sulawesi for manufacturing purpose but these wheat grains which were claimed by plant quarantine officers has contaminated by Tilletia indica. This typical fungal, which infects wheat grains, is categorized as pest plant quarantine A1. The scientific investigation, afterwards, was conducted and recommended that the import of wheat grains should be allowed to be manufactured as long as their bran residual must be burnt or ruined in order to prevent fungal dispersal in the indigenous crops on the farming-land in South Sulawesi.*
Keywords: Pest Risk Assessment, Pest Quarantine A1, Tilletia indica, wheat grains

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INTRODUCTION

Last two years, on April 2011, The Letifindo Company imported wheat grains from Pakistan to Makassar, south sulawesi. There were 259 metric tons of wheat grains in five numbers of containers. The authority of plant import and export namely, Plant Quarantine office in Makassar, a month after importing wheat grains, has addressed a letter of investigation to Plant Protection Department, Faculty of Agriculture, Hasanuddin University, about *Tilletia indica* infested the wheat grains which it is obvious pest A1 category. Regarding with the rule of No.14 of 2002 if each carrier has contaminated by pest of A1 category regarding to plant quarantine, it must be destroyed. Based on this, the officer of plant quarantine gave several options to the importer, Letifindo Company, whether they received a recommendation letter of what five of containers contaminated. Consequently, all containers of owning the importer, which contain wheat grains from Pakistan, must be burnt. However, the importer claimed that the investigation should be done more accurate from another research independent in order that an outcome of investigation is more reliable.

In order to prevent import of wheat grains from Pakistan, moving illegally another place and mixing with other wheat grains, the plant quarantine officer in Makassar imposed sealing off containers until obtaining the scientific result. Although fining sealing off all containers, they are allowed to import another wheat grains from Australia due to having letter of allowance from the authority. It is possible to do as long as under the authority inspection.

REGULATION

Regarding with the wheat grains case that imported 5 containers and company data we need to explore following below:

1. The importer, Letifindo Company, was purchased 129.480 M/TON in number of wheat grains with Number 110000000058 through PETER CREMER (S) GMBH.
2. PETER CREMER (S) GMBH pointed out the shipment of MV NEDLLOYD HUDSON V.1107, in 20th March 2011 from port of Qasim Pakistan to port of Makassar Indonersia with total number of 5 cargos of ship following Table 1;

Tabel 1. Cargo Pakistan Milling Wheat

SR NO	Container Number	Seal Number	Net Weight
01	GLDU5439677	ML-PK0575940	26.610 M/TONS
02	MSKU7123614	ML-PK0576072	24.410 M/TONS
03	MSKU2794630	ML-PK0568699	26.190 M/TONS
04	MSKU2742621	ML-PK0568700	25.860 M/TONS
05	MSKU7808315	ML-PK0568692	26.410 M/TONS
Amount			**129.480 M/TONS**

3. The authority assessed and released a CERTIFICATE OF QUALITY AND QUANTITY by Interfek Pakistan (Pvt) Ltd of which was sampled 2.5 kg of grains onto 3 containers for seal number 125197, 125198 and 125199 following Table 2;

Table 2. Data analysis

S.#	Parameter	Results
1	Test Weight	76.3
2	Protein (DMB)	12.59
3	Moisture	11.90
4	Foreign Matter (Non edible)	1.39
5	Foreign Matter (Edible)	2.88
6	Wet Gluten	26.76
7	Falling Number	37.5
8	Shrunken & Broken kernel	2.76
9	Damaged Grains	1.88

4. Prior to shipping the wheat grains import from Pakistan, the shipment must be treated by pest risk assessment based on the government rule with number 14 in 2002 about plant quarantine. This is clearly to note that in the chapter II, the rule is about criteria of plant quarantine, article 2 (a) is that Carrier media entered into the territory of Republic of Indonesia must be completed with a Plant Health Certificate from the country of origin or country of transit for plant and parts of plant, except for carrier media grouped in other goods. Therefore, quarantine treatment is a fumigation of wheat import. The Company of Jawad Fumigation Corporation in Pakistan fumigated the grains by using gas of Phostroxin substance following the concentration of 1.1 TAB/M3. In terms of implementing fumigation, the government of Republic Pakistan through Ministry of Food and Agriculture Department of Plant Protection released a letter of Phyto-sanitary Corticated.
5. The letter of plant quarantine office in Makassar was addressed to the director of Letifindo Company about the necessity of pest risk assessment for five containers of imported wheat grains (129.48 tones). It was, therefore, claimed that firstly, the wheat grains infested significantly a dangerous fungal *T.indica* afterwards the grains were isolated into potato dextrose agar media and investigated in microscope. This standard procedure is legally in doing pest risk assessment. Obviously, this fungal is categorized in the pest A1 and group 1 of crop pest and disease of quarantine. Well-growth fungal *T. indica* into the PDA media was indicated that the grains infested noticeably. Fungal identification under the microscope aimed to know whether it was still growing or not during the shipment. In terms of what literature review shows that the fumigation, with using Phospine substance as pretreatment in the Port of Pakistan before exporting, should have killed Teliospores of *T. indica*.
6. Following the letter of Plant Quarantine Office in Makassar about reassessing the imported wheat grains of 5 containers (129.48 tons), It was belief strongly that the grains have contaminated by *T. indica*, which is categorized into pest and disease plant quarantine of A1. This fungal has not found in

ecosystem of Indonesia yet. Therefore, **based on quarantine rule of No 14 of 2012**, the grains should be banned to import or destroyed, indeed. According to Pasal 20 article 2 (a) that when the restriction and observation treatments are conducted and still unsterilized from pest and disease Plant quarantine A1, aiming at point 1, The authority can ruin those carrier media..

7. According to the law of number 3 that importer can point out the qualified research institution in conducting trial adjustment, what so called ***Uji banding***, under the supervising Plant Quarantine officers in BBKP Makassar. However, it is quite confusing because, in terms of **regulation No 14 of 2002**, there is no adjustment of trial of pest risk assessment as quarantine recommended previously. Therefore, as research institution, Plant Protection Department, Faculty of Agriculture, University of Hasanuddin, argue that the adjustment research of pest risk assessment which was suggested by Plant Quarantine Makassar is as similar as a scientific letter of pest risk assessment related to pest and disease quarantine 1A. Moreover, fining the ruin of grains import, which infected by pest and disease A1 of plant quarantine, is not obviously described in this law as long as import of grains is used for need for seed conservation under territory of Republic of Indonesia and for food as well. As a consequence, the importer asked for Department of Plant Protection with well-qualified research and educational institution to cope with its serious problem. That is why a letter of scientific investigation released.

LITERATURE REVIEW: CAUSE OF KARNAL BUNT DISEASE ON WHEAT

Bioecology of *T. indica*

US Department of Agriculture, in 1996, announced recognizing the Karnal bunt disease in Arizona region therefore Plant Quarantine regulation has banned wide spread of wheat grains and supervised distribution of wheat grains in several border countries such as Arizona, New Mexico, and Texas. Mark [1] claimed that a half of wheat fields was planted came from the wheat producer countries.

The significant impact of *T. indica* infestation was losing wheat yield productions that infected wheat shifted their color and released bad smell when were consumed [2]. Although plant disease experts pointed out that lost yield production and quality of wheat, it was not noticeable effect [3]. However, karnal bunt disease was vital politically and economically in the international wheat trade and *non-tariff trade barrier* [4]. Aujla [2] reveled that approximately 70% of lost income in the international trade was caused by the Karnal burn disease. So, American Phyto-pathological society, in 1996, suggested strongly fining a zero tolerance of every wheat import. This regulation was regarding with the pathogen of *T. indica* which causes karnal bunt disease of cereals namely wheat, barley, rice, corn, sorghum, and oat can be prevented effectively under seed treatment of chemical substance, resistant variety, and planting management. These treatments are believed that although seed treatment seems to have not effective to avoid the pathogen, other trials can be implemented such as plant nursery and resistant variety to control typical soil born pathogen. However, this zero tolerant regulation for wheat import is not supported enough by scientific data for phyto-sanitary standard because this objection can be truly applied to control effectively the seed born pathogen or karnal bunt disease with combining between an alternative crop management and good seed quality in spite of zero tolerant regulation.

Symptom Disease and Life Cycle Pathogen

Commonly, this seed born pathogen is difficult to indentify in field therefore we need the accuracy investigation into wheat fields. In all night the wheat, for instance, seem to be infected **several bulir** with clear symptom following Figure 1. Fungal *T. indica* is a group of Basidiomicetes with Ustilaginales order. The teliospores appearance is to seem like dust and dark so that is why it is well known as smut fungi (Figure 2).

Teliospore of *T. indica* (see: figure 2) has diploid (2N), thick wall, and mature globose or subglobose with average diameter of 35 micro μm (roughly 22 – 49 μm). Teliospores resist against unsuitable weather and endure for 2 - 5 years in the soil. According to [5] that, the fungal is a typical soil born pathogen and has less ability to invade from grain to crop (non seed born pathogen). Teliospore of *T. indica* is dormant soon after panicle formation of wheat and it needs to germ for nine months.

Smilanick [6] pointed out that when wet condition the dormant stage occurs and teliospore emerges in surface ground. From this, nucleous of teliospores undergoes meiosis step and then following mitosis development. As a result, each pro-mycelium or a basidium grows and produces approximately 180 basidiospores (1N) known primer sporidia which are released onto the tip of basidium [5]. In common, according to Sminlanick [6] that a teliospora, is emerging within 2 mm in dept of the ground, is not able to come out in the surface soil when it invades its host. A primary sporadia has 64-79 μm long and 1.6-1.8 μm in width. It then emerges, grows and develops miselium. From this, the secondary spore is developed in large number. The secondary spore has 11.9 and 13.0 μm long and approximately 2 μm in width [7].

Infectious process occurs initially when both primary and secondary sporidia emerge in the glum of wheat grain and is spread out by splashing water. As a result, sporidia spread out in the soil surface and reach in the wheat grains growing. Then, the initial sporidia are delivered by assisting splashing water onto leaves surface near the ground. From this, they emerge and develop in the leaf surface. New sporidia releasing are carried by both water and wind towards the twigs, reaching at the grain flowering [8]. When flowering stage comes during 2-3 weeks, the crop becomes susceptible in karnal bunt disease because the pathogen infects particularly on the pericarp but it has not developed within endosperm of the grain [9]. When the grain is ripe, teliospora develop in the large number (see figure 3). In the ripening time, the small number of these teliospora carries with wheat harvest while the large number of spores remains in the ground as inoculums source. According to Gill [10] that, in Punjab India, the large number of teliospora of *T. indica,* which isolate from the soil, are found within the crop infecting with 2 x 10^3 to 5 x 10^3 per cm^3 soil.

Figure 1. Typical symptom of Karnal bunt onto panicles. To make sure whether the wheat is infected we need to harvest whole seeds because pathogen disappears when it is still in the plantation [8].

Figure 2.Teliospores of *T. indica* can be observed under the microscope. Mature teliospores seem to have from light to dark brown. The average diameter of teliospores is 35µm and appears easily to see into water treatment when rinsing infected grains [8].

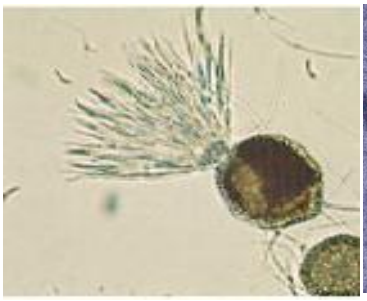

Figure 3.Teliospora whose emerging releases basidium (pro-mycelium) with own mainsporidium is long and narrow [8].

Figure 4. The Karnal bunt disease symptom has various stages from pre-infection like narrow spots of what grain surface is covered teliospore. Seed seems to havehole appearance after teliospora covers when grain is separated from the straw [11].

Epidemiology of Karnal Bunt Disease

The vulnerability of wheat from the karnal bunt pathogen is when the wet weather in the crop flowering stage because the pathogen emerges and develops [5]. The pathogen develops properly with optimum temperature, which is range of 15-20°C. Furthermore, humidity plays vital role to occur the karnal bunt disease because the development of teliospora needs more or less 82% of humidity and waterdrop in the leaf surface. When the rainfall occurs for 2-3 weeks flowering stage, soon after that the grain infects [5]. The more humid environment is the more severity incidence of grains occurs. Bonde [8] however, claimed that larger number of areas is rarely found therefore significant lost yield production is never

reported. Diekman [12] added that, in common, karnal bunt disease is not constraint wheat farming because it never causes significant lost yield in large areas in US.

Pathogen Dispersal

Teliospora survives within the soil in long term (5 years) in the laboratorium condition. Dispersal of pathogen in longer distance occurs when wheat contamination of this pathogen carries with transport as well as spreading out by wind [5]. Distribution of this disease in California, for instance, occurs when train loads the grains in Mexico and travels to California [13]. Another effective way in dispersing the Karnal bunt disease is by cattle and birds. They eat the contaminated grains and the pathogen survives in salivary canal. When they move on another place, they spread out with their feces [14]. On the other hand, Bonde [8] argued that teliospora of pathogen, commenced to attack new wheat field, was still questionable and never reported.

The Original Hosts of T. indica

The original hosts of *T. indica* are bread wheat (*Triticum aestivum*), durum wheat (*Triticum durum*) and triticale wheat as result of cross pollination between Secale (rye) and *Tritico secale* [15].

Pest Risk Assessment for *Tilletia indica*

Plant quarantine officers, 20th May 2011, in Makassar revealed imported wheat grains infected by *T. indica*, from Pakistan where is one of suspected karnal bunt disease countries, but the disease has not found in Indonesia yet. Therefore, *T. indica* categorized pest and disease plant quarantine A1 and Letifindo Company's warehouse is sealed in 6th May 2011. Following pest risk assessment as legal procedure aims to know risk development rate of seed born pathogen and spreading in new areas if these wheat import is allowed to manufacture for wheat flour and bran purposes in South Sulawesi.

What has *T. indica* found in South Sulawesi?

It has never been exist.

What is the Quarantine Status for *T. indica*?

Tilletia indica is categorized pest and disease quarantine IA.

What is the original host of *T. indica*?

The original host of this pathogen is wheat family namely bread wheat (*Triticum aestivum*), durum wheat (*Triticum durum*) and triticale (*Tritico secale*). These kinds of wheat is only *T. aestivum* is growing in South Sulawesi but it is planted with rigorous areas. Growing *T. aestivum* aims at research purpose in South Sulawesi, particular upland area (Malino area), is fewer number of areas and spread places as well. In South Sulawesi, the main crops are rice and corn which they are not alternative hosts of karnal bunt pathogen.

Any Possibilities of *T. indica* Exit in South Sulawesi?

The pathway of *T. indica* entering into Indonesia, particularly in the ecosystem of South Sulawesi seems to have possibility through wheat import from origin countries whose endemic karnal bunt disease. Dispersal of this pathogen, however, in South Sulawesi through shipping in short distance such as loading from warehouse in Makassar to manufacture industry is an obvious thinner infectious occurrence. Stein [16] pointed out that the gains which have infected and was loaded along side the wheat plantation was obvious risky and might have potential distribution massively because teliospora can release during the shipment and handling process. I do believe that in case of wheat imported from Pakistan through, the importer, Letifindo Company has a thin risk in dispersing *T. indica* in the wheat plantation in the environment of South Sulawesi. There are several strong reasons of the distance and location of wheat plantation. In Makassar, there is no wheat plantation so that during loading and handling process it is safer, reaching at 3 km distance from Port and storage of Letipindo Company. Another reason is why distribution of karnal bunt disease is impossible in this ecosystem that there is no wheat plantation around the trip. Although the pathogen release during the transport, the pathogen can not survive for long term due to hostless.

In addition, the amount of teliospores of pathogen in reaching farm-land with long distance is less possibility to survive. It is true that according to Bechtel [17], over 99% of teliospores broke down and was unsuccessful emerging and developing when the grains were manufactured to become wheat flour and bran products. The dispersal of this kind of pathogen, therefore, seems to have no possibility in south Sulawesi ecosystem. He added that never the spore reported and found in the last product, flour and bran, so that it remained in only smaller percentage (0.60 %) of survival, finding in the bran. As usual, the bran is mainly used to make cattle food. Smaller amount of spores, carrying into the cattle food and becoming feces, seems to have good in its condition but there is no scientific evidence that it can reinfect wheat [8].

Can Teliospora of *T. indica* Survive and Disperse on The South Sulawesi Ecosystem?

In the theory, a disease can only occurs if synchronize between three main factors. First of all, pathogen is virulent, environment is suitable, and last the host is vulnerable. Therefore, *T. indica* is found on the

wheat grains infestation. It can be assumed that the pathogen is virulent enough to develop disease. Environmental factor mainly affect to develop the disease. The high humidity, between 15 to 20 ^{o}C is the best condition for infecting the host as well as South Sulawesi's climate such as upland in Malino area. Although both factors of suitable environment and virulent pathogen are present, the development of disease can not occur without the host. As a result, the pathogen becomes extinct. Similarly, the wheat grain, that imported from Pakistan and contaminated by *T. indica*, has thinner opportunity to spread out in South Sulawesi because its host is far less available host and far way between wheat plantation and the Storage (about 70 km), giving possibility the spores to remain in the soil. However, the spore is dormant for 3-5 years and need wheat as a host to germinate and to form life cycle for producing new generations. Therefore, the possibility of developing and dispersal of pathogen is thinner because wheat plantation is not developed massively in South Sulawesi.

Commonly, a pathogen is able to disperse in new areas or countries, develop properly, and then survive, if its host is available. The host is used to survive and develop, and fix its life cycle [18]. According to FAO in 2003 that an international standard procedure for *pest risk analysis* (PAR) must be implemented with following an initial observation, which aims to check whether the host is present or not, regular monitor of its stable population on the potential risk of areas and its life cycle.

T. indica has restricted host of only wheat family while in South Sulawesi wheat plantation is not many areas, just focusing in upland with smaller scale area. Therefore, the development of karnal bunt disease caused by *T. indica* is fruitless and meaningless. Never it been reported the infectious *T. indica* in both wheat plantation, rice and main crops of South Sulawesi since a letter of scientific report was written. Although the spore can endure for 3-5 year in the soil, reported by [8], the spore can not fix its life cycle as long as the less available host and finally spores become undeveloped.

What is negative effect of *T. indica* for South Sulawesi economy?

When dispersal of *T. indica* in South Sulawesi, the lost yield may decline wheat production in both quality and quantity but it is insignificant. It is because the ability of pathogen to destruct in field condition is lesser. In terms of potential loss of economy is significant if the implementation of rule of **"zero tolerance"** towards *T. indica* in wheat. When the wheat grains import from Pakistan is prohibited to manufacture or is burnt due to grain infection reason, it makes importer enormous loss of economy. In addition, imposing an obligation of wheat industry to import from another country like Australia, whose *T.indica* free, makes the expenditure's company is costly and cost production become significant rise because the cost of fining row materials are higher than from Pakistan.

In 2002, Animal and Plant Health Inspection Service (APHIS) dan Plant Protection and Quarantine (PPQ) US Department of Agriculture shifted the regulation on the Karnal bunt disease. PPQ with drew the rule in wheat producer areas under supervising PPQ in Arizona, New Mexico, and Texas, which covered roughly 200,000 ha. The reasons behind with drawing the rule in these areas were *T. indica* free in farming in terms of scientific survey and observation for long terms. These areas, also, have not planted on wheat for last five years and even ever planted wheat for one season after implementing the quarantine regulation. Even if these areas were planted wheat plantation for long terms, after assessing, still the areas were *T indica* free. Therefore, wheat plantation was deemed to have less risky in providing inoculum sources for wheat plantation centers in these countries.

United Stated is one of biggest producer wheat countries in the world with export value of million dollars every year, the government with drew the rule of plant quarantine related to kernel bunt disease in wheat producer centers and they believed that those areas had lesser risky to infect kernel bunt pathogen. So that, if US allows wheat regulation, Indonesia should rethink and be more flexible and adaptable on the wheat regulation of pest and disease quarantine A1, but still implement comprehensively pest risk assessment.

Relationship Between Tilletia and Mycotoksin

Nine types of toxins are reported onto grains and food but species of *T.indica* and other *Tilletia* have not found to release one of toxins. Therefore, grains infected by *T. indica* should not be worried. In manufacturing process, the fungal toxin can be identified in flour of wheat or bran products.

CONCLUSIONS

Development and distribution *T. indica* from wheat grains imported by Letifindo Company in ecosystem in South Sulawesi is unlikely least opportunity following by;

1. Wheat grains imported by Letifindo aims to manufacture to become wheat flour and bran, which the manufacturing process can surely devastate over 99% of grains infected by teliospores of *T. indica* if still remains in the bran. Furthermore, the distance between port and Letifindo's warehouse is quite

far, about 3 km and there are not wheat plantations. In upland, there are about 70 km from Letifindo's warehouse where wheat are growing.
2. Others claim that spores of *T. indica* is risky if typical spore spreads out in the ecosystem from warehouse during processing and threats both wheat yield production and other indigenious crops such as rice and corn. However, this doubness can be refused through scientific assessment.The least risk occurs on the other crops because only wheat is its host.
3. Karnal bunt *T.indica* and another genera Tilletia are mycotoxinless as last product of wheat manufacturing is safer from human health.

RECOMMENDATIONS

Research Institution and Agricultural High Education:

Research institution and high education should conduct basic research that can be a model of pest risk assessment for *T. indica* in Indonesia. the vital issue of this study is to survey the existence of *T. indica* on the wheat plantation in South Sulawesi, to assess virulence of *T. indica* on the cereal crop and wild grass in same family with (Graminae) rice and corn in South Sulawesi, to collaborate various investigations such as climate, weather, and suitable development and distribution of *T. indica* in South Sulawesi, to know effectiveness in manufacturing process wheat grains in order to reduce the number of teliospores in farming of South Sulawesi.

Plant Quarantine Office

Plant Quarantine Institution should assess pest risk treatment properly to all kinds of quarantine pest and disease of quarantine 1A including *T. indica*. This trial should be supported by scientific research in comprehensive way based on Indonesia's ecosystem. The result of pest risk assessment (PRA) should become a fundamental assessment to implement **pest risk management and mitigation** and shift an alternative regulation of "**zero tolerance**".

Letifindo Company

a. If the wheat grain import by Letifindo Company is being sealed off by the Plant Quarantine Institution of Makassar and is allowed to be manufactured for wheat flour and bran purposes, its strongly believe that the bran, which was from grains and infected *T.indica*, should be burnt despite selling for cattle food. All of equipments in manufacturing process involved should be sterilized and cleaned before using next processing. In order to being done appropriately with standard procedure, all procedures are under the investigation and observation's Plant Quarantine officers.
b. In the future, fulfillment of wheat need in this region, import of wheat grains, should not be imported from endemic pest and disease quarantine A1 such as *T. indica*. If necessary, following the government regulation.

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