- BUREAU OF AGRICULTURE AND FISHERIES STANDARDS -

TECHNICAL BULLETIN

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Title	**Terminal Report: Efficacy Testing of Thyme Guard 23% SL Against Rice Blast (*Magnaporthe oryzae), * Sheath Blight *(Rhizoctonia solani)* Sheath Rot *(Sarocladium oryzae)* False Smut *(Ustilaginoidea virens)* of Rice (*Oryzae sativa)* RC 160 Variety**
Introduction	Rice is one of the political commodities of the Philippines because most Filipinos depend on rice grains as their source of staple food (Intal & Garcia, 2005). It has enormous cultural significance that goes far beyond basic nutrition. Rice cultivation has been part of Filipino culture for centuries. It is often associated with community activities and festivals, such as planting and harvesting ceremonies. These events highlight the importance of cooperation, unity, and shared values among Filipinos. If there is a scarcity of rice grains, Filipinos will suffer food shortages eventually causing chaos to the populace (Yang, 2019). Due to its importance in the Filipino diet, ensuring a stable and affordable supply of rice is essential for food security (David & Huang 1996). Historically, issues related to rice availability and prices had social and political implications in the Philippines. Ensuring an adequate supply of rice at reasonable prices is crucial for maintaining stability and preventing unrest. The government's efforts to manage rice production, distribution, and pricing have a direct impact on the well-being of the population. The government struggles to supply rice due to epidemics of rice diseases, which can impact food security and crop availability. Effective management of these diseases is crucial for maintaining agricultural productivity, reducing hunger and malnutrition, and achieving food independence. Researchers can develop novel disease-resistance strategies and sustainable farming methods. Since the earliest writings, plant diseases have been understood. Fossil evidence suggests that plant disease may have existed 250 million years ago. The Bible specifically mentions diseases like rusts, mildews, and blights in the books of Haggai 2:17 and Amos 4:9. These diseases have caused famine and other significant changes in the economies of nations since the beginning of recorded history (Kelman et al., 2023). In less developed nations, where access to disease-control methods is limited and annual losses of 30 to 50 percent for major crops are not uncommon, crop loss due to plant diseases can also lead to hunger and starvation. For those who rely on the crop for food, losses can be catastrophic in some years when they are much higher. Major food crop disease outbreaks have caused famines and mass migrations throughout history (Kelman et al., 2023).

One of the most harmful diseases to affect rice is rice blast. Up until the tillering stage, a leaf blast infection can kill seedlings or plants, a severe leaf blast infection reduces leaf area for grain fill at later growth stages, lowering grain yield. In cases of severe infection, leaf blast can kill rice plants at the seedling stage and reduce yield (IRRI Rice Knowledge Bank, 2023).

Rice sheath blight, which has affected between 120,000 and 190,000 hectares in Japan and has been reported to have caused a yield loss of as much as 20%, is of growing concern for the production of rice, especially in intensified production systems. It has also been reported to have caused a yield loss of 6% in tropical Asia (IRRI Rice Knowledge Bank, 2023).

For sheath rot, Taiwan has experienced a yield loss of 20–85%, while Viet Nam, the Philippines, and India have experienced losses of 30–80%. When an infection happens during or after the booting stage, before the panicle emerges, it is most destructive (Health diagnosis, 2023).

False smut causes chalkiness and can reduce 1,000-grain weight. It also causes a reduction in seed germination of up to 35%. In damp weather, the disease can be severe and losses can reach 25%. In India, a yield loss of 7–75% was observed (IRRI Rice Knowledge Bank, 2023).

Utilizing synthetic fungicides in controlling rice diseases offer several advantages for agricultural management (Law, et al., 2017). Firstly, synthetic fungicides are often highly effective at rapidly suppressing disease outbreaks, helping to minimize crop losses and ensure food security (Gianessi & Reigner, 2005). Secondly, they provide a targeted approach, specifically addressing the pathogens responsible for rice diseases without harming the crop itself (CropLife International, 2023). Thirdly, synthetic fungicides are relatively easy to apply and integrate into existing agricultural practices, requiring minimal adjustments to conventional farming methods (FAOSTAT, 2023). Fourthly, their efficacy can be predictable and consistent, offering a reliable solution to farmers facing recurrent disease challenges (OECD-FAO, 2012). Finally, synthetic fungicides can contribute to increased crop yield and improve overall farm profitability when deployed strategically and judiciously (CropLife International, 2023).

The conventional method usually uses synthetic fungicides in controlling the pathogenic fungus, but with their effort in suppressing the inoculum of the pathogen, the harvested products were contaminated with pesticide residues, and worst of all, heavy metals were detected on the products that were heavily applied with synthetic fungicides (Asiah, et al., 2019).
To solve this problem, it is important to find an alternative fungicide that is environmentally friendly and easily degrades its residue in the

environment. Several beneficial characteristics of fungicides derived from botanical sources make them useful for managing disease in agriculture. They typically degrade more quickly and have fewer aftereffects than synthetic alternatives, they frequently have a lower environmental impact (Deresa, E. M., & Diriba, T. F., 2023). Botanical fungicides generally reduce the risks associated with chemical exposure, making them safer for human health and beneficial to non-target organisms (Yoon, et al., 2013). These organic substances have a variety of modes of action, pathogen resistance—which is a growing worry with synthetic fungicides—is less likely to arise. By fostering biodiversity and ecosystem health, botanical fungicides can support sustainable agricultural practices. Many botanical sources are accessible locally, which may lower production costs and give farmers, particularly in areas with limited resources, a practical option (Ngegba, 2022). With all the advantages of the fungicides derived from botanicals only a few are utilized, and a handful of commercialized ones are not registered to the competent authority. Unregistered and untested formulation of botanical fungicides can pose a threat to users because its efficacy is not scientifically tested by the competent authority.

It is timely that a new generation of essential oil extracted from thyme, Thyme Guard 23% SL is a 100% biodegradable broad-spectrum contact liquid bactericide, fungicide, and insecticide for use on all organic crops: food and nonfood. Its molecular structure makes it efficient, striking the cellular membrane of bacteria and fungi without generating resistance (Thyme Guard, 2023). Thyme Guard 23% SL has proven its high efficacy against diseases like Botrytis, Fusarium, Powdery Mildew, Downy Mildew, Citrus Canker, Citrus Greening-HLB, Fire Blight, and many others. Thyme Guard 23% SL also has insecticidal effects on sucking insects such as scales, white flies, psyllids, and mites (Thyme Guard, 2023). The antibacterial, insecticidal, and possibly antifungal properties of thyme are believed to exist (Felman, 2018). Alves et al. (2019) evaluate the antifungal properties of Portuguese thyme species, *Thymus camphoratus,* and *Thymus carnosus*, in traditional medicine, revealing their superior efficacy against *Cryptococcus neoformans* and dermatophytes. Moghaddam and Mehdizadeh (2020) found that hydrodistillation essential oil from *Thymus vulgaris* aerial parts contains 45 compounds, with thymol and ρ-cymene being the main components, effectively treating *M. phaseolina*. Ziyat et al. (2021) revealed that active ghassoul formulations, including Gh-A-thyme, and Gh-A-thymol, demonstrated significant antifungal activity against *Penicillium sp*., with over 75% inhibitory potential and retention over time. Thyme oil effectively reduces fungi colonization on drinker surfaces and litter in broiler houses, suggesting that essential oils may be effective prevention methods against fungal aerosols (Witkowska et al., 2016). Omidbeygi et al. (2007) found that thyme essential oils effectively inhibited *Aspergillus flavus* growth in culture medium and tomato paste. Thyme oil, containing thymol, is known for its antibacterial and antifungal properties, and its antioxidant activity may help prevent tooth decay as a mouthwash ingredient (Bulzoni, 2018). Thyme oil is safe because it is used in mouthwashes and liniments to kill germs (Thyme, 2021).

Conducting efficacy trials of thyme oil for controlling rice diseases is essential to assess its potential as a sustainable and environmentally friendly solution. Such trials provide scientific evidence of thyme oil's effectiveness in preventing or mitigating rice diseases, which could reduce the reliance on chemical pesticides. By evaluating the impact of thyme oil on disease incidence and severity, researchers can determine its practical application in real-world agricultural settings. This information is vital for farmers, as it empowers them with an alternative method that could enhance crop yield and quality. Furthermore, successful efficacy trials could pave the way for promoting integrated pest management practices and fostering more ecologically balanced rice cultivation systems.

Objective

The general objective of efficacy trials was to generate efficacy data of Thyme Guard 23% SL *against Rice Blast (Magnaporthe oryzae); Sheath Blight (Rhizoctonia solani); Sheath Rot (Sarocladium oryzae); and False Smut (Ustilaginoidea virens) on Rice*, as a prerequisite for product registration with DA-BAFS. Specifically, the trials aimed:

1. to determine the efficacy of Thyme Guard 23% SL; and
2. to determine the effective dosage/s of Thyme Guard 23% SL.

Methods

1. **Efficacy Trial Period and Locations**
   The efficacy trials were conducted in Poblaction, Kabacan, North Cotabato (Location 1), and Maligaya, Matalam, North Cotabato (Location 2).

2. **Target Crop and Pests**
   The target crop was rice, RC 160 variety, with its intermediate reaction to blast, bacterial leaf blight, and green leaf hopper. The variety is susceptible to tungro, moderately susceptible to brown plant hoppers, resistant to yellow stem borer, and moderately resistant to white stem borer (PhilRice, 2022). The target pests were as follows:

   a. **Rice Blast** *(Magnaporthe oryzae)* can infect the rice plant's leaf, collar, node, neck, and parts of the panicle, and occasionally the leaf sheath is all susceptible to it (IRRI Rice Knowledge Bank, 2023). Low soil moisture, frequent and protracted rain showers, and cool daytime temperatures are all factors that contribute to its occurrence (MU Extension, 2023). Large day-night temperature differences that result in dew forming on leaves and generally cooler temperatures in upland rice favor the growth of the disease (Muimba-Kankolongo, 2018). Rice can develop blast at every stage of growth (Boddy, 2016). However, as plants mature and build up adult plant resistance to the disease, the incidence of leaf blasts tends to decline (TNAU, 2023).

b. **Sheath Blight** *(Rhizoctonia solani)* is a fungal disease caused by *Rhizoctonia solani*. Infected leaves senesce or dry out and die more rapidly, young tillers can also be destroyed (Senapati et al., 2022). As a result, the leaf area of the canopy can significantly be reduced by the disease. This reduction in leaf area, along with the diseased-induced senescence of leaves and young infected tillers are the primary causes of yield reduction (IRRI Rice Knowledge Bank, 2023). Sheath blight occurs in areas with high temperatures (28–32°C), high levels of nitrogen fertilizer, and relative humidity of crop canopy from 85–100% (ASP, 2023). High seeding rate or close plant spacing, dense canopy, disease in the soil, sclerotia or infection bodies floating on the water, and growing of high-yielding improved varieties also favor disease development (MU Extension, 2023).

c. **Sheath Rot** *(Sarocladium oryzae)* lowers grain yield by causing unfilled seeds and sterile panicles, as well as delaying or stopping panicle emergence. Sheath Rot also lowers grain quality by rotting panicles and discoloring grains (IRRI Rice Knowledge Bank, 2023). Sheath Rot can be found in most of the world's rice-growing nations, especially in rice ecosystems that are fed by rain, and it is more common during wet than dry seasons (Bigirimana, et al., 2015). Its prevalence rises with higher planting densities and in plants that serve as entry points for the fungus, in the form of wounds and injuries brought on by insects like stem borers at the start of panicles. The fungus usually causes the uppermost leaf sheaths that surround young panicles to rot, which delays or prevents the emergence of the panicles (Health diagnosis, 2023). This can happen in regions with heavy nitrogen fertilizer application, high relative humidity, and temperatures between 20 and 28 °C when crops are beginning to mature (IRRI Rice Knowledge Bank, 2023). The uppermost leaf sheath enclosing the young panicles is where the typical sheath rot lesion begins. It appears as an irregular or oblong spot with a center that is either brownish-gray or gray with brownish-reddish edges (Afifah et al., 2020). Typically, several spots are seen, which enlarge, combine, or grow together until they can cover the majority of the leaf sheath. Panicles may partially emerge or remain inside the sheath. A lot of mycelium, a whitish powdery fungal growth, may be visible on the surface of affected leaf sheaths. Undeveloped panicles rot, and the florets change color from red-brown to dark brown (TNAU, 2023).

d. **False Smut** *(Ustilaginoidea virens)* causes chalkiness of grains which leads to a reduction in grain weight (Khanal, et al., 2022). It also reduces seed germination. Temperatures between 25 and 35 °C and high relative humidity (>90%) are conducive to the occurrence of the disease. Rain, high humidity, and soils with high nitrogen content also favor disease development. Wind can spread the fungal spores from plant to plant. False smut is visible only after panicle exertion. When the plant is in the flowering stage, it can infect it. Individual rice grains that are infected with false smut turn into a mass of spore balls on plants. (Fan et al., 2019). Individual rice grains become a mass of spore balls when a plant becomes infected with false smut. These spore balls start orange and eventually turn greenish-black as they mature. Individual rice grains that are affected by false smut mutate into a mass of spore balls on plants. When these spore balls mature, they change from orange to a greenish-black color (IRRI Rice Knowledge Bank, 2023).

3. Efficacy Trial Design and Layout

The efficacy trials were laid out randomly with four replications. There were 10 sampling units per treatment plot in each replicate. Plot measured 3 m x 4 m providing four replicates, with a total of 48 sqm per treatment. A distance of 1.5 m between plots and 1.5 m between blocks were employed in the trials. A uniform seeding rate of 144 g/plot was used and planted as direct seeding.

4. Treatment Protocols

The dosage and frequency of treatment application are shown in Table 1. The treatment sequences are chronological to avoid treatment contamination.

Table 1. Dosage and frequency of treatment application

Treatment	Dosage (ml/L)	Frequency
T1	Untreated	
T2	1.0	Every 7 days (11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81), days after seeding (DAS). 11 cycles of application.
T3	2.0	
T4	3.0	

Pre-treatment assessment of disease in rice was gathered before the application of treatment to determine the disease pressure in the area. Efficacy evaluation of the treatments was done three and six days after application (DAA). Uniform spray deposits on the leaf surface were ensured by conducting spray calibration before applying the treatments. The application time and monitoring of disease was done early in the morning or not earlier than 4:00 in the afternoon.

5. Cultural Management Practices

Land Preparation

To soften the soil, the field was irrigated one week before the first plowing. All the dikes were repaired to keep the paddy full of water while also flooding the habitat of the rodents. Soft soil is ideal for the optimum growth and development of the root of the rice plant. In the first tillage, a rotavator drawn by a 35 hp tractor was employed. For the second and third tillage, harrowing was employed at a seven-day interval. Draft animals were employed to harrow the field for initial leveling. The field was leveled properly to achieve optimum control of the weeds and the golden apple snail (GAS). A properly prepared rice field contributes to faster rice plant recovery and hastens the decomposition of weeds and plant residues.

The seeds were soaked for twenty-four hours after being dried for two to three hours. Before and after soaking, the seeds were cleaned to get rid of any contaminants. Every six hours, the water was replaced. Up until the root appeared, the seeds were incubated at 30 °C. To speed up germination, the seeds were kept moistened and well-ventilated. The seeds were sown after the roots started to come out, 10 g/m2 of seeds were evenly sown. The experimental plots were irrigated six days after sowing. The water level was gradually increased to 1-2 cm, which facilitates controlling the growth of the weeds. Golden apple snail was controlled by handpicking.

Fertilizer Management

The application of fertilizer is important in improving crop growth and yield. For the side dress, 120 g/plot of urea and 14-14-14 fertilizer were applied at 14 DAS, respectively. A 60 g/plot of 21-0-0 and 0-0-60, respectively, was applied at 30 DAS for the top dress.

6. **Sampling**

   The sampling unit was determined randomly. The number of infected plants and their severity was measured using ten randomly tagged sample plants per plot. Randomization in experimental settings reduces bias and ensures uncontrollable factors don't influence results. It assigns treatments to subjects, minimizing confounding variables, achieving a representative sample, and enabling accurate inferences. Tagging experimental plants helps track and identify individual plants, ensures data attribution, monitors progress, records changes, collects analysis data, traces anomalies, and interprets results.

7. **Analysis of Results**

   The data gathered were subjected to analysis by comparing the difference of mean of treatments against untreated control. The

	standard percent comparison is set in the *Philippine National Standard (PNS) – Organic Bio-control Agents (OBCA) – Microbials and Botanicals – Minimum requirements (PNS/BAFS 182:2016).*
Data Gathered	1. **Disease Severity.** Disease severity was assessed at 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81 DAS (at weekly intervals) until one week before harvest. Total of 11 applications. The disease severity (DS) in percentage was determined by rating the disease severity as a value in the interval scale. DS (%) is calculated by dividing the sum of the class frequency by the rating class score by the total number of plants multiplied by the maximum disease index and then multiplying the result by 100. Efficacy evaluation of the treatments was done three and six DAA. 2. **Disease incidence.** Disease incidence was assessed at 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, and 81 DAS (at weekly intervals) until one week before harvest. The infected plants from 10 randomly tagged sample plants per plot were collected and recorded and the average percentage of infected plants was determined by the number of plants with damage divided by the total number of sample plants and then multiplied by one hundred. Counting the actual number of infected plants was done and converted to the % disease incidence. Efficacy evaluation of the treatments was done three and six DAA. The following diseases were gathered: **a. Rice Blast** ***a.1. Leaves.*** Ten randomly tagged sample plants per plot were used to determine the incidence and severity of infected plants with rice blast disease. The DA-BAFS OBCA Manual's (First Edition) Annex A - Table 4 rating system was used to determine the average percentage of infected plants' severity. A disease incidence scale was created by converting the actual plant count that was infected into a percentage. From growth stage 0 to 5, reading was done. Three and six days after application were when the treatments' efficacy was assessed. ***a.2. Node and neck.*** The incidence and severity of rice blast disease-infected plants were determined by randomly tagging ten sample plants per plot. The DA-BAFS OBCA Manual's (First Edition) Annex A - Table 3 was used to rate the severity of the average percentage of infected plants. A percentage disease incidence scale was created by taking the actual count of the infected plants and converting it. Reading began in growth stages seven through nine. Three and six days after application were used to evaluate the treatments' effectiveness.

b. Sheath Blight

Collect and record infected plants from 10 randomly tagged sample plants/hills and the average sheath and leaf infection was determined following the rating scale of the DA-BAFS OBCA Manual's (First Edition) Annex A - Table 5. Reading was taken at growth stages five to nine. Efficacy evaluation of the treatments was done three and six days after application.

c. Sheath Rot

Gather and document infected plants from ten randomly tagged sample plants or hills. The average number of infected tillers was calculated using the DA-BAFS OBCA Manual's (First Edition) Annex A - Table 6 rating system. Five to nine growth stages were studied in reading. Efficacy evaluation of the treatments was done three and six days after application.

d. False Smut

Ten randomly tagged sample plants or hills were used to gather and record infected panicles. The average degree of damage was then calculated using the DA-BAFS OBCA Manual's (First Edition) Annex A - Table 7 rating system. Growth stages seven through nine were the ideal times to start reading. Three and six days after application were used to assess the treatments' efficacy.

Results & Discussion

1. **Percent control against Rice Blast (*Magnapothe oryzae)* severity.** The treatment and its percent control against Rice Blast severity, which passed the PNS for OBCA (PNS/BAFS 182:2016), is the Thyme Guard 23% SL at 3 ml/L at 63.78% and 63.80% for trial locations 1 and 2, respectively.

2. **Sheath Blight *(Rhizoctonia solani)***
   The treatment and average percent control against Sheath Blight incidence and severity, which passed the standard efficacy set in the PNS/BAFS 182:2016, are shown in Tables 2 and 3.

Table 2. Percent control against sheath blight incidence

Treatment	*Location 1*	*Location 2*
Thyme Guard 23%SL at 3 ml/L	89.28%	65.40%

Table 3. Percent control against sheath blight severity

Treatment	*Location 1*	*Location 2*

Thyme Guard 23%SL at 2 ml/L	72.50%	58.82%
Thyme Guard 23%SL at 3 ml/L	91.73%	73.06%

3. **Sheath Rot *(Sarocladium oryzae)***
   The treatment and average percent control against Sheath Rot incidence and severity, which passed the standard efficacy set in the PNS/BAFS 182:2016, are shown in Tables 4 and 5.

Table 4. Percent control against Sheath Rot incidence

Treatment	***Location 1***	***Location 2***
Thyme Guard 23%SL at 2 ml/L	83.27%	50.58%
Thyme Guard 23%SL at 3 ml/L	92.25%	68.54%

Table 5. Percent control against Sheath Rot severity

Treatment	***Location 1***	***Location 2***
Thyme Guard 23%SL at 2 ml/L	83.28%	59.69%
Thyme Guard 23%SL at 3 ml/L	93.25%	73.72%

4. **False Smut *(Ustilaginoidea virens)***
   The treatment and average percent control against False Smut incidence and severity, which passed the standard efficacy set in the PNS/BAFS 182:2016, are shown in Tables 6 and 7.

Table 6. Percent control against False Smut incidence

Treatment	***Location 1***	***Location 2***
Thyme Guard 23%SL at 1 ml/L	54.17%	68.43%
Thyme Guard 23%SL at 2 ml/L	100.00%	89.58%
Thyme Guard 23%SL at 3 ml/L	100.00%	100.00%

Table 7. Percent control against False Smut severity

Treatment	***Location 1***	***Location 2***
Thyme Guard 23%SL at 2 ml/L	100.00%	71.90%
Thyme Guard 23%SL at 3 ml/L	100.00%	64.39%

Conclusion and Recommendation

Conclusion and Recommendation

The product Thyme Guard 23%SL was able to meet the efficacy standards set in the PNS/BAFS 182:2016 at ≥50 percent and as required by the Department Circular (DC) 05, Series of 2020 *(Guidelines on the Registration of Organic Bio-Control Agents Producers and Products)* as amended by DC No. 01, Series of 2021, against Rice Blast, Sheath Blight, Sheath Rot and False Smut of rice. Thus, the product is recommended to apply for product registration with the DA-BAFS.

Practical Implication

The efficacy results suggest that the product, Thyme Guard 23%SL, can be used to control the incidence and severity of major diseases of rice with the dosage and frequency as shown in Table 8.

Table 8. Diseases, dosage, and frequency of application

Target Disease	Dosage/Rates (Thyme Guard 23%SL)	Frequency and Method of Application
Rice Blast	3 ml/L water	Every 7 days, foliar spray
Sheath Blight	2-3 ml/L	Every 7 days, foliar spray
Sheath Rot		
False Smut		

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Annex

-Photo Documentation

Figure 1. Field layout of the Research Area, Location 1: Kabacan, Cotabato

Figure 2: Field layout of the Research Area, Location 2: Matalam, Cotabato

Figure 3: The plastic barrier was used to mitigate the spray drift.

Figure 4: The untreated plot was severely damaged by the leaf blight at Location 1: Kabacan, Cotabato.

Figure 5. The treated plot with Thyme Guard 23% SL is less damaged compared to the untreated plot at Location 1: Kabacan, Cotabato.

Figure 6. The treated plot with Thyme Guard 23% SL is less damaged compared to the untreated plot at Location 2: Matalam, Cotabato.

Figure 7. The untreated plot was severely damaged by the leaf blight at Location 2: Matalam, Cotabato.

Figure 8. Rice Leaf Blast symptoms.

Figure 9. Sheath Blight symptom.

Figure 10: Sheath Rot symptoms.

Figure 11: False Smut symptoms.