Title	**Terminal Report: Efficacy Testing of Thyme Guard 23% SL Against Early Blight *(Alternaria solani)*, and Late Blight *(Phytophthora infestans)* of Tomato *(Solanum lycopersicum)***

Introduction

Tomatoes *(Solanum lycopersicum)* are economically significant worldwide due to their versatility, widespread consumption, and support of various industries (Lizardo et al., 2022). Tomatoes are one of the most widely grown and economically important vegetable crops on the planet (Liberato, 2017). They are grown in a variety of countries, employing millions of farmers and agricultural workers (Kimeu, 2021).

Tomatoes are rich in essential nutrients like vitamins (e.g., vitamin C and vitamin K), minerals (e.g., Potassium), and antioxidants (e.g., Lycopene) (Sass, 2022). Their widespread consumption promotes public health by providing access to nutritious food (Collins, et al., 2022). Tomatoes are a fundamental ingredient in a wide range of food products, including sauces, soups, ketchup, salsa, and various processed foods (Swartzendruber, 2018). The food industry benefits greatly from the demand for tomato-based products, which drives sales and employment in this sector (Neto et al., 2017).

Tomatoes are a crucial agricultural commodity with far-reaching economic significance (Gorme et al., 2017). They contribute to employment, international trade, food processing, and various related industries while also promoting health and nutrition (Marin, et al., 2020). The economic importance of tomatoes extends across the globe and benefits both rural and urban economies (Tabbun, et al., 2018).

Tomato Early Blight, caused by the fungal pathogen *Alternaria solani*, is a destructive disease that affects tomato plants during their early growth stages (University of Minnesota Extension, 2023). Symptoms include small, dark brown to black lesions on the lower leaves, often with a "bull's eye" pattern (North Carolina State, 2023). As the disease progresses, the affected leaves may turn yellow, and the lesions can expand, leading to leaf tissue withering and death (University of Maryland Extension, 2023). In severe cases, it can also affect tomato fruit, resulting in dark, sunken lesions, and reducing fruit quality and marketability (Mississippi State University, 2023). Early Blight spreads through airborne spores, which can be carried by wind, raindrops, or human activities (University of California Extension, 2023). The disease thrives in warm and humid regions and can survive in soil for extended periods, increasing the risk of recurrence (West Virginia University, 2023). Regular inspection and early detection are crucial for prompt control measures and successful tomato cultivation (Tabbun, et al., 2018).

Tomato Late Blight is a fungal disease that can cause significant crop loss and damage to tomato plants. Symptoms include small, dark green to black, irregularly shaped spots or lesions on the leaves, which enlarge and turn brown or dark brown. As the disease progresses, entire leaflets or leaves may become infected, and lesions spread across the leaf surface. Infected fruit may develop dark, irregularly shaped lesions, causing the tomato to rot, become mushy, and emit an unpleasant odor. Stem lesions can also be affected, leading to wilting and eventual plant collapse. Late Blight spreads rapidly, especially in cool, wet weather conditions, and is more prevalent in regions with these conditions. Symptoms become more pronounced overnight or during high humidity, with a white, fuzzy appearance on infected areas due to sporulation.

Tomato Early Blight *(Alternaria solani)* and Late Blight *(Phytophthora infestans)* are fungal diseases that significantly reduce tomato yields, leading to reduced fruit production and lower revenue for farmers (Tabbun, et al., 2018). It also incurs additional expenses like fungicide costs, labor, and increased water usage. Infected tomatoes may have blemishes, reduced size, or lower quality, affecting market prices or rejection by processors. Severe outbreaks can affect the overall tomato market, increasing consumer prices and potentially reducing consumption. To mitigate these effects, research, education, and the development of disease-resistant tomato varieties are essential. These methods can help control the disease and reduce production costs, making tomato blight a significant economic issue for farmers and the agricultural industry.

The application of synthetic fungicides to plant disease management offers various advantages for agricultural management. (Law et al., 2017). Synthetic fungicides are often very successful in quickly controlling disease outbreaks, which lowers crop losses and guarantees food security (Gianessi &

Reigner, 2005). Fungicide provides a focused strategy that spares the crop damage while precisely targeting the pathogens that cause diseases (CropLife International, 2023). The application and integration of synthetic fungicides into current agricultural practices is comparatively easy, requiring only slight modifications to conventional farming techniques (FAOSTAT, 2023). Their effectiveness can be steady and predictable, giving farmers a dependable way to deal with ongoing disease problems (OECD-FAO, 2012).

The conventional method usually uses synthetic fungicides to control the pathogenic fungus, but in their effort to suppress the pathogen's inoculum, 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).

Synthetic fungicides have been widely used in agriculture to combat fungal diseases that threaten crop yields and quality. While they have undoubtedly contributed to increased agricultural productivity, they also have several significant disadvantages and drawbacks. While synthetic fungicides have played an important role in protecting crops from fungal diseases, their drawbacks are significant and cannot be overlooked. The negative environmental impact, resistance development, health risks, and long-term consequences for soil health.

An eco-friendly substitute fungicide that readily breaks down its residue in the environment needs to be created to solve this problem. Plant-based fungicides are beneficial for managing diseases in agriculture because of their many beneficial qualities. Compared to synthetic alternatives, they usually degrade more quickly and leave fewer aftereffects, which lessens their impact on the environment (Deresa, E. M., & Diriba, T. F., 2023). In general, botanical fungicides lower the hazards of chemical exposure, improving non-target organism health and safety for human health (Yoon et al., 2013). The development of pathogen resistance, an increasing concern with synthetic fungicides, is less likely with these organic substances due to their multiple modes of action. By fostering ecosystem health and biodiversity, botanical fungicides can be beneficial. Local access to a wide variety of botanical sources may reduce production costs and provide farmers, especially in resource-poor areas, with a viable option (Ngegba, 2022).

It is timely that a new generation of thyme essential oil, Thyme Guard 23% SL (2023), is a 100% biodegradable broad-spectrum contact liquid bactericide, fungicide, and insecticide for use on all organic crops: both food and nonfood. Due to its molecular makeup, it can effectively attack bacterial and fungal cell membranes without causing resistance. Thyme Guard 23% SL (2023) has demonstrated its high effectiveness against diseases like Botrytis, Fusarium, Powdery Mildew, Downy Mildew, Citrus Canker, Citrus Greening-HLB, Fire Blight, and many others. Additionally, Thyme Guard 23% SL has insecticidal properties against sucking insects like scales, white flies, psyllids, and mites (Thyme Guard, 2023). Thyme is thought to have antibacterial, insecticidal, and possibly antifungal properties (Felman, 2018). *Thymus camphoratus* and *Thymus carnosus*, two species of Portuguese thyme used in traditional medicine, are evaluated for their antifungal properties by Alves et al. (2019), who find that they are more effective against dermatophytes and *Cryptococcus neoformans*. Moghaddam and Mehdizadeh (2020) found that hydro-distillation essential oil from Thymus vulgaris aerial parts contains 45 compounds, with thymol and ρ-cymene being the main components, effectively treating *M. phaseolina*. Active ghassoul formulations, such as Gh-A-thyme and Gh-A-thymol, showed significant antifungal activity against *Penicillium sp.*, with over 75% inhibitory potential and retention over time, according to Ziyat et al. (2021). According to Witkowska et al. (2016), thyme oil effectively reduces the amount of fungi that colonize drinker surfaces and litter in broiler houses, indicating that essential oils may be useful as preventative measures against fungus aerosols. Thyme essential oils effectively slowed the growth of *Aspergillus flavus* in tomato paste and culture medium, according to Omidbeygi et al. (2007). As a mouthwash additive, thyme oil, which contains the antibacterial and antifungal compound thymol, may help prevent tooth decay due to its antioxidant activity (Bulzoni, 2018). Due to its use in liniments and mouthwashes to eliminate germs, thyme oil is safe (Thyme, 2023).

Validating thyme oil's potential as a natural remedy for controlling fungal diseases in agriculture requires conducting efficacy trials for the substance. These trials provide farmers with reliable data on its performance, enabling them to make informed decisions about incorporating thyme oil into their pest and disease management strategies. These trials also help promote the adoption of organic fungicides like thyme oil, reducing the use of synthetic chemicals harmful to ecosystems. By establishing optimal application methods and dosages, thyme oil can be used efficiently and economically. Scientific validation of thyme oil's efficacy can expedite the regulatory approval process, making it more accessible to farmers. Consumer demand for organic and environmentally friendly products is growing, and efficacy trials build consumer confidence in thyme oil-treated produce. In the long term, conducting efficacy trials for thyme oil supports the transition to sustainable agricultural practices, benefiting both the environment and the agricultural industry.

Objective

The general objective of efficacy trials was to generate efficacy data of Thyme Guard 23% SL against Early Blight and Late Blight of tomato, as a prerequisite for product registration with DA-BAFS. Specifically, the trials aimed to:

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

Method

1. **Efficacy Trial Period and Location**
   The trials were conducted from Nov 2022 to March 2023 at Magallon, Mlang, Cotabato (Location 1), and from January 2023 to April 2023, at Kapatagan, Digos City, Davao del Sur (Location 2).

2. **Target Crop and Diseases**
   Tomato Diamante Max F1 is a heat-tolerant hybrid tomato that produces well and exhibits moderate levels of resistance to tomato leaf curl virus (ToLCV) and bacterial wilt. Fruits can be stored and transported well because they are tall, firm, and round. The target diseases are as follows:

   a. **Early Blight**
   One of the most prevalent diseases affecting tomatoes is Early Blight, which can appear all year round. When susceptible tomato cultivars are used and the weather is right, it can have a significant negative impact on yield and affect leaves, fruits, and stems. The incidence and severity of damage percentage were gathered and recorded based on the manifestation of the symptoms.

   b. **Late Blight**
   Late Blight, a fungus that infects plants, is primarily caused by infected transplants, volunteer plants, and related weeds, with spores carried by rain during cool, rainy weather. Data on the incidence and severity of damage

percentage were collected and recorded based on the occurrence of the symptoms.

3. Efficacy Trial Design and Layout

There were 10 sampling units per treatment plot in each replicate as shown in Figure 1. The trials were laid out randomly with four replications.

The plots measured 3 m x 4.5 m providing a total of 54 square meters per treatment. In each plot, tomato seedlings were planted at a distance of 50 cm between hills, and 150 cm between rows, or 24 plants per plot.

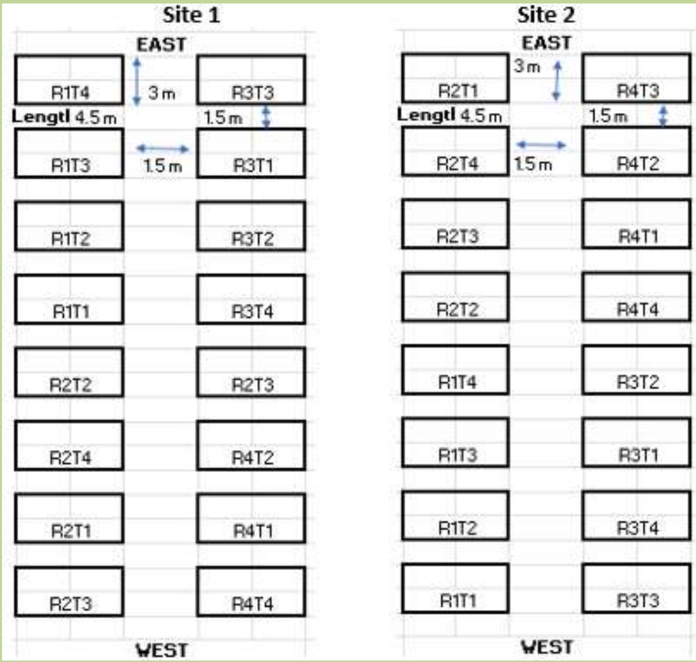

Figure 1. The trial's experimental design for the two locations.

4. Treatment Protocols

Table 1 displays the dosages and frequency of treatment applications. To prevent treatment contamination, the treatment sequences are chronological in order.

Table 1. Dosages and Frequency

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	

Before treatment application was administered, pre-treatment data on tomato disease was acquired to ascertain the level of disease pressure on the plant. Days after application (DAA), the efficacy was assessed three and six days later. Before administering the treatments, spray calibration is done to guarantee uniform spray deposits on the leaf surface. The application was done early in the morning, or no later than 4:00 in the afternoon, for the disease incidence and severity assessment.

5. Cultural Management Practices

a. Land Preparation

The soil was plowed and harrowed one month before transplanting. Furrows were made at 1.5 m. Twelve sacks of chicken dung were applied per 1,200 square meters at 20 days before transplanting.

b. Seedling Bag Preparation

The bags were filled with soil and placed in an area fully exposed to sunlight. Drenched the bags with 16-20 fertilizer (100 g) dissolved in 20 L of water. After the fertilizer was drenched with fertilizer, tomato; 1-2 seeds were placed at the center of the bag.

c. Transplanting

Selected healthy seedlings with 3-5 leaves 3-4 weeks after seedling emergence were transplanted. One seedling was transplanted per hill spaced 50 cm apart. Seedlings were transplanted in the morning. Newly transplanted seedlings were watered immediately and covered with banana bracks. Missing hills were replanted 5-7 days after transplanting.

d. Fertilizer Management

An initial side dressing of one tablespoon (10 g) of 16-20-0 was applied three weeks after the plants were transplanted, 6–8 cm from the base of the plants. A second side dressing was done two weeks later, using one tablespoon of the combined fertilizer. Every two weeks, fertilizer was administered.

e. Water Management

The tomato plants were watered if there was no rain within three days after the last rain occurred. For some instances that there was no rain in one week the tomato plants were irrigated with an interval of three days.

f. Pest Management

Insect pests were controlled by applying Aktrine 0.6 SL as an insecticide. The application of insecticide was not mixed with Thyme Guard. The insecticide was applied if the insect pest population was present in the field. Weeds were controlled by hand weeding.

g. Harvesting

Harvesting was done when the fruit started to change its color to red yellowish color. This was done in five primings.

6. Sampling

Ten plants per plot were randomly tagged in the inner rows of the plot and randomly tagged in the inner rows of the plot for a sampling of tomato disease. A pretreatment assessment was gathered to determine the disease pressure in the sample plant.

7. Analysis of Results

The data gathered were subjected to analysis by comparing the difference of the mean of treatments against the untreated. The standard percent comparison is set in the *Philippine National Standards (PNS) Organic Bio-control Agents (OBCA) – Microbials and Botanicals – Minimum requirements (PNS/BAFS 182:2016).*

Data Gathered

Disease Severity. The disease severity for Early and Late Blights was computed using the established procedure and rating as prescribed by DA-BAFS OBCA Manual, as shown in Table 2. The efficacy evaluation of treatments was done three and six days after application.

Table 2: Rating Scale for Disease Severity

Scale	*Percent Area Infected*
0	None
1	1-5%
3	6-12%
5	13-25%
7	26-50%
9	More than 50%

Disease Description:

1. **Early Blight**. The symptoms that manifest on older leaves, infections first appear as tiny brown spots that quickly grow larger. The lesions are typically surrounded by a yellow halo. A single lesion can kill an entire leaf if it grows larger and coalesces. Collect and record infected plants from 10 randomly tagged sample plants/hills and determine the average fruit and leaf infection following the rating scale.
2. **Late Blight**. Tomato leaf Late Blight lesions can range in shape from round to irregular and have a brown dry center with a pale green halo when the weather is

dry. Collect and record infected plants from 10 randomly tagged sample plants/hills and determine the average fruit and leaf infection following the rating scale.

Results & Discussion

1. **Disease Severity. Percent Severity Control Against Early Blight.** The treatments and their percent (%) control against the severity of Early Blight disease, which passed the standard efficacy set by the PNS/BAFS 182:2020, are shown in Table 3.

Table 3: Percent Severity Control Against Early Blight

Treatment	Location 1	Location 2
T3 - Thyme Guard 23% SL at 2 ml/L	53.02%	62.17%
T4 - Thyme Guard 23% SL at 3 ml/L	65.40%	80.92%

2. **Disease Severity. Percent Severity Control Against Late Blight.** The treatments and their % control against the severity of Late Blight disease, which passed the standard efficacy set by the PNS/BAFS 182:2020, are shown in Table 4.

Table 4: Percent Severity Control Against Late Blight

Treatment	Location 1	Location 2
T3 - Thyme Guard 23% SL at 2 ml/L	62.54%	51.98%
T4 - Thyme Guard 23% SL at 3 ml/L	68.57%	61.56%

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%, and as required by the Department Circular No. 01, series of 2021. Thus, the product is recommended for label expansion with DA-BAFS.

Practical Implication

The efficacy results suggest that the product can be used to reduce the severity of Late Blight (*Phytophthora infestans)* and Early Blight (*Alternaria solani)* in tomato plants with the following dosages, as shown in Table 5.

Table 5. Dosage and Frequency of Applications

Disease	Dosage and Frequency of application
Early blight and Late Blight disease	2-3 ml/L at weekly interval, 11 cycles of application

Researchers and

Researcher

Company Profile	**JOSEPH O. CASTILLO** BAFS-OADRS-AR-058 DA-BAFS Certified Researcher DA-Special Order No. 065, series of 2022 ***Company*** **AKTIV MULTI TRADING CO. PHILS. INC.** Door 205, GRDC Bldg., Km. 7 Lanang, Brgy. Rafael Castillo, Davao City
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Annex

-Photo Documentation

Figure 2. Tomato Early Blight disease taken at Location 2: Kapatagan, Digos City, Davao del Sur.

Figure 3. The purpose of the plastic barrier was to reduce the spray drift.

Figure 4. Early Blight stem infection on untreated plots at Location 2: Kapatagan, Digos City, Davao del Sur.

Figure 5. Late Blight infection at Location 1: Magallon, Mlang, Cotabato.

Figure 6. The treatment application of Thyme Guard 23% SL aided with plastic barrier to mitigate the spray drift at Location 1: Magallon, Mlang, Cotabato.

Figure 7. Severe infection of Early Blight at Location 2: Kapatagan, Digos City, Davao del Sur.

Figure 8. Late Blight infection on untreated plot at Location 1: Magallon, Mlang, Cotabato.

Figure 9. The treated plot, located at Location 2: Kapatagan, Digos City, Davao del Sur, exhibits uniform tomato plant growth due to its protection from the Early Blight, with Thyme Guard 23% SL affecting fewer plants.

Figure 10. The untreated plot of the tomato plant that the height is not uniform because it was affected by the Early Blight, taken at Location 2: Kapatagan, Digos City, Davao del Sur.

Figure 11. Experimental set up at Location 1: Magallon, Mlang, Cotabato.

Figure 12. Experimental set up at Location 2: Kapatagan, Digos City, Davao del Sur.

Figure 13. Spray drift was lessened by using the plastic barrier at Location 2: Kapatagan, Digos City, Davao del Sur.

Figure 14. Late Blight severity damage on untreated plots at Location 2: Kapatagan, Digos City, Davao del Sur.

Figure 15. Late Blight incidence and severity damage on the plot treated with 3 ml Thyme Guard 23% SL at Location 2: Kapatagan, Digos City, Davao del Sur.

Figure 16. Early Blight incidence and severity damage on the plot treated with 3 ml Thyme Guard 23% SL at Location 1: Magallon, Mlang, Cotabato.