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Trichoderma-MEDIATED PHENOLIC CONTENT ENHANCEMENT OF *Origanum vulgare* L.

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ABSTRACT – The use of medicinal plants constitutes an integral aspect of the Philippine primary healthcare system. The high cost of medicines in the country limits adherence and increases reliance on low-cost herbal medication such as *Origanum vulgare* L. (oregano). This plant is a common herb used by locals as traditional and alternative medicine owing to the antioxidant and antimicrobial properties of its phenolic components. In line with this, *Trichoderma* Microbial Inoculant (TMI), comprising three local strains (*T. ghanense, T. pseudokoningii,* and *T. harzianum),* was applied as a soil drench to oregano transplants to observe the responses in growth and phenolic content. Results showed that within 49 days of planting oregano cuttings, at $p < 0.05$, no significant differences in growth parameters were observed between the untreated and treated oregano. *Trichoderma* did not exert any observable adverse effects on the plant. However, a significant increase was observed in the total phenolic content, with the treated plants (0.04 mg Gallic Acid / g dried oregano leaf) exhibiting four times more phenols than the control (0.19 mg Gallic Acid / g dried oregano leaf). Qualitative assessment through thin-layer chromatography showed that carvacrol and thymol (the main phenolic compounds responsible for the antimicrobial and antioxidant properties of oregano) exhibited a slightly more intense band in the treated versus the untreated control. The findings show that *Trichoderma* triggers an enhanced defense response in oregano without adversely affecting its growth, indicating an uncoupling of the natural defense-growth trade-off in plants. This effect is crucial, as it demonstrates the potential of *Trichoderma* inoculation to enhance the medicinal value of plants by increasing bioactive, medically relevant defense metabolites, without adversely affecting the plant's growth. To our knowledge, this is the first study conducted in the Philippines to investigate the interaction between *Trichoderma* and *O. vulgare*.

Keywords: Origanum vulgare, oregano, Trichoderma, phenol, uncoupling of plant growth-defense trade-off

INTRODUCTION

Traditional medicine is utilized for treatment, diagnosis, prevention of illnesses, and maintenance of human well-being. It encompasses health practices that utilize various materials and techniques, including those involving the use of plants (Fokunang et al., 2011). According to the World Health Organization (WHO, 2022), traditional medicine, including herbal medicines, is utilized by around 88% of all countries worldwide. In the Philippines, the high cost of both branded and generic drugs has

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deemed most medicinal drugs unaffordable for the lower-income brackets (Clarete & Llanto, 2017), leading to an increased reliance on herbal medicines. Hence, evidence-based research on the traditional medicinal plants has become necessary to develop safe, effective, and low-cost herbal medication in the country (Maramba-Lazarte, 2020). Thus, the Traditional and Alternative Medicine Act (TAMA) of 1997 was established with the guiding principle of "improving the quality and delivery of healthcare services to the Filipino people through the development of traditional and alternative health care." It aims to encourage scientific research on the development of traditional medicine, such as the verification and transfer of economically viable technologies in the fields of traditional and alternative healthcare.

In the country, traditional healers, especially in the provinces, have long used herbal medicines in their practice, this includes lagundi (*Vitex negundo*), sambong (*Blumea balsamifera* L.), and ampalaya (*Momordica charantia* L.). These plants belong to the 10 scientifically validated medicinal plants under the FDA and DOH (Principe & Jose, 2002). However, another commonly used herb is Greek oregano (*Origanum vulgare* L.). A study conducted by Fabie-Agapin (2020) has cited *O. vulgare* as one of the medicinal plants used by traditional healers in Pagadian City, Zamboanga del Sur, Philippines. It is traditionally used as a remedy for cough and cold and is taken as a tea (Adlawan, 2022). Despite not being included in the FDA and DOH's list of important medicinal plants, scientific studies have demonstrated the importance of its bioactivity to human health. In traditional medicine, the leaves, dried herbs, and volatile oil of oregano (Oregano Essential Oil, OEO) are commonly used.

OEOs are phytochemicals that have been extensively studied for their antioxidative activity (Leyva-López et al., 2017). Studies have demonstrated the antioxidative properties of OEO in relation to the presence of phenolic structures, such as thymol and carvacrol (Martucci et al., 2015). These antioxidants are beneficial in enhancing the immune responses in various organisms (Alagawany et al., 2020; Gholami-Ahangaran, 2021; Hashemipour et al., 2013), thereby exhibiting their potential for health improvement. A study by Burt (2004) has demonstrated that carvacrol and thymol react with free radicals, turning them into more stable products, thereby leading to the termination of radical chain reactions.

The *Trichoderma* genus is a member of the family Hypocreaceae and is a natural inhabitant of the soil and the plant's rhizosphere. These endophytic fungi are described as avirulent plant symbionts that provide nutrients, promote plant growth, control pests and diseases, and induce systemic resistance (Harman et al., 2004; Li et al., 2015; Lee et al., 2016; Martínez-Medina, 2014). *Trichoderma*'s growth-promoting properties have been observed in herbs (He et al., 2020; Rostaminia et al., 2021; Wang et al., 2020) and other plants (Cai et al., 2015; Iannucci et al., 2024; Marra et al., 2022;). Aside from enhancing a plant's primary metabolism, *Trichoderma* has also been observed to increase the secondary metabolites and active ingredients of its host plant including phenols (Gębarowska et al., 2019; Iannucci et al., 2024; Marra et al., 2022; Wang et al., 2020). In particular, the *Trichoderma* Microbial Inoculant (TMI) developed in UPLB has shown many beneficial effects on different host plants, including biocontrol, growth-promotion, better nutrient absorption, improved crop nutritional profile, increased productivity and yield, enhanced resistance to abiotic stresses (e.g., drought, heavy metals), and induced systemic resistance (Banaay et al., 2023).

Trichoderma Microbial Inoculant (TMI) commercialized as Biospark™ *Trichoderma* is a biofertilizer and biocontrol agent developed at the Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños. It consists of three local species: *Trichoderma ghanense, T. pseudokoningii,* and *T. harzianum* (AGORA UPLB, 2022). In this experimental study, the Biospark™ *Trichoderma* is inoculated into oregano cuttings via soil drenching. However, since the experiment was conducted in a field setting that includes practical constraints such as the lack of strict

control over environmental factors, unquantifiable variations are expected and therefore pose limitations on the conclusions derived. These variations include uncontrolled weather conditions, varying light intensities, and possible differences in plant water exposure. Furthermore, only one genotype of oregano was used in conducting this study which was obtained from the Bureau of Plant Industry - Los Baños National Crop Research, Development and Production Support Center (BPI-LBNCRDPSC).

The objective of this study is to determine the responses of *Origanum vulgare* L. upon inoculation with Biospark™ *Trichoderma* Microbial Inoculant (TMI) in terms of the vegetative growth and phenolic contents. Based on related studies, it is hypothesized that TMI will positively impact the growth and phenolic quantity of oregano.

In the Philippines, there is a difficulty in finding published studies examining the effects of *Trichoderma* inoculation on the phenolic content of *O. vulgare*, despite phenolic compounds accounting for most of its medicinal properties. Thus, results of this study can provide valuable insights into the ability of TMI to enhance bioactive compounds, such as phenols. This is especially important in determining a technique that can help increase the phenolic contents of herbal medicines, which have antioxidant properties, while promoting the sustainable use of biofertilizer. This research is vital to the academe, agricultural, and health sectors as it is the first study in the Philippines investigating the interactions between *Trichoderma* and *O. vulgare*.

MATERIALS AND METHODS

For this research, Greek oregano (*Origanum vulgare* L.) stem cuttings were obtained from BPI-LBNCRDPSC. On the other hand, the commercialized biofertilizer agent Biospark™ *Trichoderma* Microbial Inoculant (TMI), consisting of 10^9 spores per gram of three local strains of *Trichoderma* (*T. ghanense, T. pseudokoningii*, and *T. harzianum*) mixed in equal proportions with coconut coir dust as carrier, was obtained from the Institute of Biological Sciences (IBS), University of the Philippines Los Baños (UPLB). Pot experiments were conducted at the IBS, UPLB, from March to May 2023. Daytime temperature ranged from 26°C to 40°C, and watering of the plants was done only as necessary, depending on the precipitation. Weather conditions for the duration of the experiment were obtained from the National Agromet Station (NAS) located nearby and inside the UPLB campus, as shown in Table 1.

Table 1. Weather conditions from March to May 2023 during the conduct of the *Trichoderma*-oregano interaction experiment.

Meteorological Parameter (unit of measurement)	Month (2023)		
	March	April	May
Mean Rainfall (mm)	3.2	62.4	107.4
Mean Temperature (°C)	26.6	27.2	29.1
Mean Relative Humidity (%)	78.0	79.0	78.0
Total Sunshine Duration (min)	14,706.0	13,290.0	10,724.0

Planting and Inoculation

During the pre-experimentation, 10 equally sized pots were filled with 500g of garden loam soil (pH~6.5-7). The soil-filled pots were then randomly and equally divided into two groups (TMI-treated and control) and arranged in a completely randomized design in an open area under full sun.

Five of the 10 pots were soil-drenched with TMI according to the manufacturer's instructions, with slight modifications to adjust to the volume of soil in the pots; this served as the TMI-treated group. Three days before planting the oregano cuttings, the TMI-treated pots were soil-drenched with Biospark™ TMI at a rate of 3.9g per L of water. Two hundred (200) mL of the TMI solution was mixed with 500g of soil/pot for the TMI-treated group. Meanwhile, 200mL of water was mixed with the 500g of soil/pot for the control group. Three days later, the 10-cm oregano cuttings were planted in the pots. Fifteen (15) days after replanting, re-inoculation of TMI at the same concentration as the initial inoculation was performed in the TMI-treated group. In contrast, only water was applied to the control on the same day. All plant samples were harvested on the 49th day after replanting.

Moreover, on random days throughout the experiment, light intensities were measured using a LuxMeter mobile application to estimate the average daylight intensity. Measurements were taken in the morning (around 9:00 AM to 11:00 AM), at noon (around 12:00 PM to 2:00 PM), and in the afternoon (around 3:00 PM to 5:00 PM).

Morphological Parameters

After the harvest, vegetative growth of both the TMI-treated and control groups was assessed using selected parameters.

Leaf Area

In determining the measurement of leaf area, only true and fully expanded leaves were taken into consideration. A total of five leaves were measured per individual plant. The Leaf Area Index (LAI) was calculated using the Montgomery Equation (Montgomery, 1911):

$$LAI = kLW$$

Wherein:

k = Montgomery Parameter determined using a simple tracing method;

L = Distance from the base to the apex of the lamina;

W = Maximum distance between two points on the lamina perimeter that forms a straight line perpendicular to the axis of the leaf length.

Leaf Chlorophyll Concentration

The chlorophyll concentration at 11:00 AM, at noon (around 12:00 PM to 2:00 PM), and in the afternoon (around 3:00 PM to 5:00 PM) was measured using a Soil Plant Analysis Development (SPAD) chlorophyll meter. This took into account the average of six readings from various parts of a leaf to obtain the chlorophyll concentration per single leaf. A total of six leaves were measured per individual plant.

Root Surface Area

The plant root surface area was analyzed using RhizoVision Explorer Version 2.0.3, with root image processing settings customized as indicated in Table 2. The roots of each plant were washed with water to remove loose soil. These images were captured under the same lighting conditions against a white background and then analyzed using the software mentioned above.

Table 2. RhizoVision Explorer Version 2.0.3 settings for root image analysis.

Analysis Option	Settings	
Root type	Broken roots	
Image thresholding	200	
Invert images	False	
Keep the largest component	False	
Filter noisy components in the background	True	
Maximum background noisy component size	1	
Filter noisy components in the foreground	False	
Maximum foreground noisy component size	1	
Enable root pruning	True	
Root pruning threshold	1	
Convert pixels to physical units	True	
Dots per inch	1	
Pixel to millimeter conversion factor	25.4	
Diameter Range 1	0	2
Diameter Range 2	2	5
Diameter Range 3	5	above

Shoot Length

The shoot was measured using a ruler, starting from the stem 5 cm above the soil and beyond. Meanwhile, the plant part below the 5-cm mark was considered as part of the root system and hence, accounted for in the root density.

Dry Mass

The oregano plant samples were dried in an oven at 40°C until crisp to the touch; the parts were then weighed.

Phenolic Content

Quantification of Phenols via Folin-Ciocalteu Test

The plant extraction procedure was adapted from Khorsand et al. (2022) with slight modifications. The oregano plants were first cut from 5cm above the soil and oven-dried at 40°C. This was followed by the pulverization of the dried samples using the Wiley® mill (with a 20 mesh size). Three biological replicates for each treatment were used for extraction. Subsequently, two technical replicates per biological replicate were obtained for the quantification of phenols. For each biological replicate, 200mg of dried leaf was dissolved twice in 20mL of 80% methanol (80:20; methanol-water) shaken for 72h at room temperature The supernatant was filtered using a fluted Whatman filter paper (No. 1); this served as the plant extract.

The Folin-Ciocalteu (FC) Method, as adapted from Khorsand et al. (2022) based on Spanos and Wrolstad (1990), with slight modification, was followed in the determination of total phenolic content. The plant extract was subjected to an FC test for quantification. For the purpose of determining the total phenolic content of oregano samples, 10µL of the Plant Extract was added to 500µL of 10% Folin-Ciocalteu reagent (10:90; FC-water ratio). This was followed by the addition of 500µL of a 7.5% sodium carbonate solution. The resulting mixture was then incubated at room temperature for two hours. For each replicate, two mixtures were prepared for absorbance readings, and the resulting absorbance values were averaged to represent each replicate. After incubation, the absorbance of the solution was then read at 765nm using a spectrophotometer.

Quantification of the total phenolic content was based on the standard curve of 0.025mg/mL, 0.05mg/mL, 0.10mg/mL, 0.15mg/mL, 0.20mg/mL, 0.25mg/mL, 0.30mg/mL, 0.35mg/mL, 0.40mg/mL, 0.45mg/mL, and 0.50mg/mL of gallic acid dissolved in 80% methanol. The 0mg/mL blank was prepared by mixing 10µL of 80% methanol with 10% FC reagent and 7.5% sodium carbonate. The same blank was used for both the control extract and the TMI-treated extract, as well as for the gallic acid standard curve. Total phenolics were reported as mg gallic acid equivalent (GAE)/g dry weight and calculated as follows:

$$Total\ phenolic\ content\left(\frac{mg\ standard}{g\ sample}\right) = \frac{concentration\left(\frac{mg}{mL}\right) x\ volume\ of\ sample\ (mL)\ x\ DF}{weight\ of\ sample\ (g)}$$

Qualitative Appraisal of the Essential Oil Profile via Thin Layer Chromatography

The qualitative detection of thymol and carvacrol components in the oregano leaf essential oil was determined using the method described by Wagner and Bladt (1996). One gram (1g) of powdered, dried oregano leaves was extracted with 10mL of dichloromethane via shaking for 15 minutes. The suspension was then filtered, and the clear filtrate was evaporated to dryness. The residue was then dissolved in 1mL of toluene, and 30µL of the resulting solution was spotted on a Merck® TLC silica gel 60 F254 plate. The Mallinckrodt® Thymol (Hazelwood, Missouri) standard was spotted side-by-side with the TMI-treated and control oregano leaf extracts.

The TLC plate was developed using a solvent system composed of toluene and ethyl acetate (93:7), designed to separate essential oil components. After the plate had been developed, it was sprayed with the Vanillin-Sulfuric (VS) reagent: first with 10mL of a 1% ethanolic vanillin solution, followed immediately by 10mL of 10% ethanolic sulfuric acid. The plate was heated at 110°C for 5min and evaluated under visible light. A characteristic red zone at Rf ~0.55 confirms the presence of thymol/carvacrol. Before spraying and subjecting it to heat, the plate was also observed under UV-254 to

confirm the presence of essential oils in the sample, characterized by quenching or the appearance of black bands on a green-fluorescing plate. On the other hand, the blue, green, and gray fluorescence in UV-365 confirms the presence of other essential oil components.

Statistical analysis

The data were processed using PAST4 (Paleontological Statistics 4) Software, in which a two-sample t-test was performed with a significance level of $p < 0.05$. Shapiro-Wilk Normality Test and F Test for Equal Variances were also employed before the actual t-test.

RESULTS AND DISCUSSION

The microflora of soil, which encompasses both pathogenic and plant growth-promoting microorganisms, plays a crucial role in influencing plant growth and development (Arora & Mishra, 2016; Janvier et al., 2007). These beneficial microbes play a vital role in the plant's growth by enhancing and regulating its nutrient availability, phytohormone production, and tolerance to stress (Lopes et al., 2021). At the same time, the host plant and beneficial microbes form a symbiotic relationship with each other, such as that of *Trichoderma*. Members of this genus colonize the roots, as they are attracted to the sucrose-rich plant root exudates, which then activate the plant's defense responses (Vargas et al., 2009).

Morphological Response of O. vulgare Inoculated with TMI

In this study, plant growth is assessed by measuring shoot length, leaf area, leaf chlorophyll concentration, root surface area, and dry mass in the control and TMI-treated *O. vulgare*. Based on the data acquired from the samples, no significant differences were observed in any of the morphological parameters between the two groups (see Table 3).

Table 3. Comparison of the average measurement of plant morphological parameters taken from the control and *Trichoderma*-treated *Origanum vulgare*.

Morphological Parameter	Average Measurement (±SE)*	
	Control	TMI-treated
Stem Length (cm)	31.00 ± 8.22	36.60 ± 6.95
Leaf Area (cm^2)**	40.21 ± 12.95	51.74 ± 8.08
Leaf Chlorophyll***	31.30 ± 1.95	30.12 ± 1.52
Root Surface Area (mm^2)	$4.70 \times 10^{10} \pm 9.88 \times 10^{9}$	$5.20 \times 10^{10} \pm 1.70 \times 10^{10}$
Overall Dry Mass (g)	2.14 ± 0.58	2.69 ± 0.26

**p>0.05; no significant differences between means for all parameters.*
*** k = 0.6407322654*
**** represented by SPAD readings*
TMI - Trichoderma Microbial Inoculant

As shown in Table 3, except for chlorophyll concentration, all other parameter measurements exhibited higher average values for the TMI-treated plants compared to the control plants. However, the differences were not statistically significant, possibly owing to high variation in the measurements. Although TMI has been known to elicit significant positive growth responses in other crops, as shown in

previous studies (Cuevas et al., 2005; Cuevas, 2006; Cuevas et al., 2012; Banaay et al., 2012; Banaay and Cuevas, 2022; Iannuci et al., 2024), *O. vulgare* in this research did not show any significant difference in growth relative to the control. Although this indicates the ineffectiveness of TMI in promoting the development of oregano, it suggests another possible role of *Trichoderma* that benefits the plant host: the uncoupling of the growth-defense trade-off.

Origanum vulgare has natural antifungal properties primarily attributed to its essential oils containing phenolic compounds such as carvacrol and thymol. These compounds can disrupt fungal cell membranes and inhibit the growth of pathogens (Moghrovyan and Sahakyan, 2024; Leyva-Lopez et al., 2017), but divert metabolites from growth to defense response. When oregano is exposed to biotic or abiotic stressors such as nutrient deficiency, pathogens, or other stressors, its defense mechanism is activated; however, this activation also leads to a growth-defense trade-off due to resource limitations (Lattanzio et al., 2009). The inhibition of growth is a cost associated with producing secondary metabolites, as the biosynthesis itself is costly, requiring the production of ATP or reduction equivalents. For these defense or signaling compounds to exhibit their function, they need to be present in relatively high concentrations, which requires the use of more resources. In addition to the biosynthesis, significant amounts of ATP are also necessary for the storage and transport of these compounds. Therefore, this makes the production of defense and signal compounds highly costly for the plant (Wink, 2010). This, then, forces the plant to sacrifice its continued growth to upregulate the production of secondary metabolites, such as phenols, which are used for defense and survival.

This phenomenon of growth-defense trade-off enables plants to adapt to varying environmental conditions by managing their metabolic costs. In this process, various phytohormones, including auxin, abscisic acid, and jasmonic acid (JA), play key roles. Stress leads to the plant's production of defensive chemicals such as JA, which in turn, activates a signaling pathway for the production of pathogenesis-related proteins. These proteins are necessary for the plant's protection against infection. However, the activation of such a defense mechanism harms the plant's growth as it may inhibit cell division and expansion (Figueroa-Macías et al., 2021).

Various studies have shown that *Trichoderma* enhances the growth of its host plant (He et al., 2020; Marra et al., 2022; Rostaminia et al., 2021; Wang et al., 2020). This is in contrast to the current findings, which show no significant difference between the control and TMI-treated samples. However, there is also no observed reduction in growth, indicating that a growth-defense trade-off did not occur.

Additionally, oregano stem cuttings were used in this study, as they are commonly found in households and provide a more accessible means of propagating the herb. However, experimenting with seeds is also worth considering. Although it takes significantly longer to grow, as the germination process must first be completed and there is a risk of non-viable seeds, it also has advantages in plant growth. One of which is its ability to produce a higher yield as the primary root grows from it (Plagron, 2023) as compared to plant cuttings, which can only form adventitious roots (Sasse & Sands, 1997). This is because adventitious roots can develop from stem or shoot tissue post-embryonically in a constitutive manner or in response to stress when subjected to cutting (Gonin et al., 2019). As observed in other plants, such as cereals and strawberries, adventitious roots have significantly lower nutrient uptake than primary roots in nutrient-depleted conditions (Steffens & Rasmussen, 2016). Thus, the difference in the effects on morphological growth might be attributed to the fact that previous studies had a more extended observation period and used seedlings (He et al., 2020) and seeds (Rostaminia et al., 2021). To account for the possible variation in growth rate caused by propagation using stem cuttings, it is best to observe the process from seed to adult stage, as this is also the recommended approach in the manufacturer's guide for plant

application with TMI. Seed coating with TMI ensures that *Trichoderma* can colonize the roots immediately and adequately prime the plant for growth and defense.

Also, only five replicates per treatment were used in the present study. While de Winter (2013) states that Student's t-test–the statistical treatment used in this paper–is still considered valid for sample sizes less than or equal to five as it does not inflate type I error rate (false-positive), it requires larger effect size to detect significant differences. As such, increasing the number of replicates may provide a more apparent distinction between differences in growth rates. This is because larger sample sizes decrease the margin of error and standard deviation, therefore, increasing the precision of results and decreasing the possibility of reporting false-negative or false-positive findings (Charlesworth Author Services, 2022).

As there are also other considerations to examine, it cannot fully be concluded that TMI indeed does not enhance the growth of oregano. However, it is worth noting that TMI did not harm the development of the host plant; instead, it even increased its total phenolic content, as observed in Table 4 and Figure 2.

Qualitative Appraisal of the Essential Oil Profile via Thin Layer Chromatography

Figure 1 illustrates the essential oil profile of *O. vulgare,* from which we can infer its components using the Thin Layer Chromatography method described by Wagner and Bladt (1996). The figure shows the TLC plate observed under visible light and under UV light at 254nm. According to the chromatographic profile observed under visible light, the red-violet zone at an R_f value of approximately 0.55 is determined as thymol and its structural isomer, carvacrol. Additionally, other compounds inferred are the blue and grey zones at R_f ~0.15 – 0.35, characterized as terpene alcohols; meanwhile, the same band color placed in the R_f range 0.7-0.8 is characterized as terpene esters. Moreover, green bands at R_f ~0.5 are inferred to be aldehydes. Lastly, other essential oil components were characterized by green and brown bands, observed at R_f values of ~0.035 and 0.12 to 0.18, respectively.

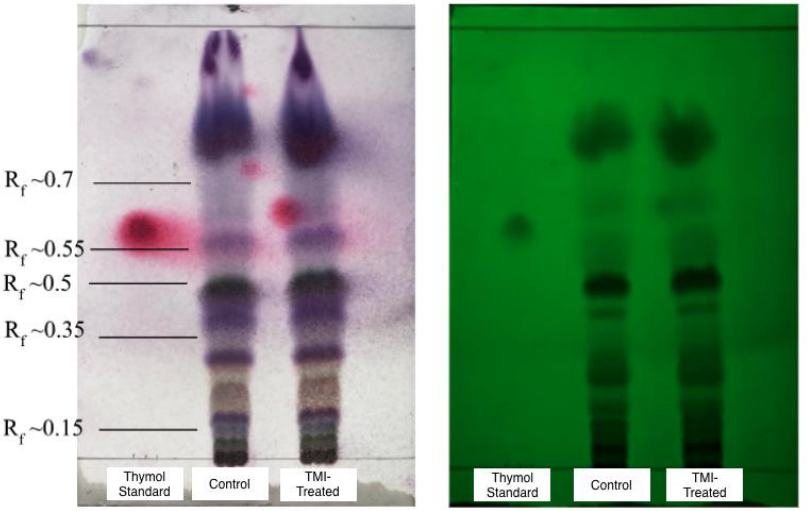

Figure 1. Thin-layer chromatograph of the essential oil profile of *Trichoderma*-treated and control *Origanum vulgare* L., observed under visible light (left) and UV-254 (right). Presence of band in R_f ~0.55 is indicative of the presence of thymol and its isomer, carvacrol.

About 90% of the phenolic compounds foĺund in the essential oil of oregano are composed of thymol and carvacrol (Pelissari et al., 2009). This makes it necessary to qualitatively determine the presence of thymol and its isomer, carvacrol, in the essential oil profile of TMI-treated oregano to determine whether or not it has the potential to produce the same beneficial effects as the non-treated oregano. This is because these two compounds are the primary contributors to the health benefits derived from the phenols in oregano essential oil. Some of the biological activities identified from oregano-extracted carvacrol and thymol are involved in food preservation as well as attributed to antioxidant, antihypertensive, and antimicrobial activities (Rathod et al., 2021). It does so by inducing cytotoxicity, apoptosis, and cell cycle arrest (Sampaio et al., 2021).

Quantification of Phenols via Folin-Ciocalteu Test

Figure 2 presents the total phenolic content of the control and TMI-treated dried *O. vulgare* leaf. It can be observed that TMI-treated samples have significantly higher total phenolic content compared to the control set up with 0.19 ± 0.08 mg Gallic Acid/g dried oregano leaf and 0.04 ± 0.03 mg Gallic Acid/g dried oregano leaf, respectively. The TMI-treated samples had four times higher phenolic content than the control.

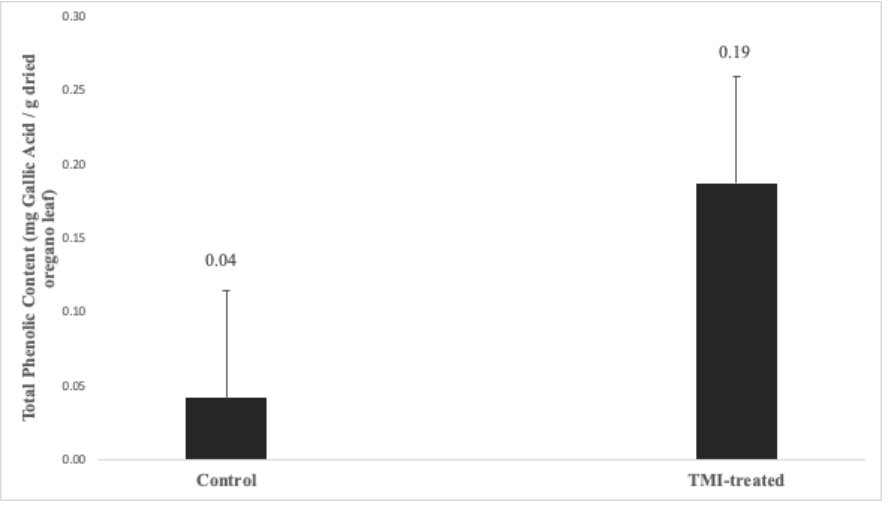

Figure 2. Comparison of the Total Phenolic Content of the control and *Trichoderma*-treated *Origanum vulgare* L.

In general, plants have defense mechanisms against pathogens; one of which is the production of reactive oxygen species (ROS) (Nanda et al., 2010). This initiates a series of subsequent reactions that lead to a dynamic defense response, inhibiting the growth of invaders, for example, by producing secondary metabolites and pathogenesis-related (PR) proteins (Singh et al., 2011). Biocontrol agents (BCAs), such as *Trichoderma* spp., can activate this "ROS gene network" to provide defense and protection (Nanda et al., 2010). Upon fungal invasion, *Trichoderma* produces and concentrates defensive compounds, such as phenols, to which it is resistant (Sood et al., 2020). These phenols exhibit potent antioxidant activity, enabling them to scavenge free radicals (Singh et al., 2011). Hence, a positive correlation exists between

phenolic content and antioxidant activity, which plays a role in protecting against ROS-mediated oxidative stress caused by pathogens (Singh et al., 2011). But, because beneficial *Trichoderma* spp. are avirulent plant symbionts (Harman, 2004), the priming of the defense response does not lead to a decrease in plant growth. Instead, a growth-promoting effect is made possible.

A hypothetical *Trichoderma* interaction proposed by Hermosa et al. (2013), based on the model proposed by Kazan and Manners (2012), involves a mutually antagonistic crosstalk between Gibberellic Acid-Jasmonic Acid (GA-JA) signaling and DELLA (aspartic acid-glutamic acid-leucine-leucine-alanine proteins) JASMONATE ZIM-domain or simply DELLA-JAZ. Here, instead of the growth being inhibited during defense activation, the trade-off is being offset by the presence of *Trichoderma*. The reduction of plant ethylene (ET) signaling caused by *Trichoderma*'s production of 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) causes an increase in GA levels; meanwhile, abscisic acid (ABA) is reduced. The GA increase then promotes the destruction of DELLA protein, a key negative regulator of GA signaling that acts immediately downstream of the GA receptor (Eckardt, 2007). Therefore, the GA-dependent degradation of DELLA protein subsequently leads to growth promotion (Yoshida et al., 2014). Typically, during the growth process, there is a suppression of plant defenses since the MYC2—the main downstream effector required for induced systemic resistance—transcription factor activity is inhibited due to the binding of jasmonate ZIM-domain (JAZ) proteins. However, *Trichoderma* also produces indole-3-acetic acid (IAA), which stimulates the ACC synthase for the biosynthesis of ET, subsequently triggering the increase in ABA synthesis. This also results in a decrease in GA levels, thereby preventing the degradation of DELLA protein and leading to growth inhibition. As the DELLAs are no longer degraded, they will compete with MYC2 for binding to JAZ—the negative regulator of JA signaling—resulting in the activation of the signaling and defenses by jasmonic acid (JA). In this hypothetical *Trichoderma* interaction, inoculation allows for the plant growth-defense trade-off to be alleviated, as *Trichoderma* IAA also contributes positively to salicylic acid (SA) signaling in plants, compensating for the suppression of growth.

Additionally, the activation of JA is also involved in the biosynthesis of phenolic compounds, as JA enhances the activity of phenylalanine ammonia-lyase (PAL); thereby activating the phenolic biosynthetic pathway–also known as the phenylpropanoid pathway–and increasing phenol content. This observation is observed in various plants, such as romaine lettuce and shrubs (Kim et al., 2007; Mendoza et al., 2018; Pàrween & Jan, 2019). Therefore, as this is the general mechanism of plants for their defense, this may explain the upregulation of phenolic contents in the TMI-treated oregano.

These phenolics play a crucial role in plant defense, acting as insecticides, natural animal toxins, protective agents, and inhibitors against invasive organisms. The synthesis of these phenols is triggered when potential pathogens are recognized by plant pattern recognition of pathogen-associated molecular patterns (PAMP), consequently activating the PAMP-triggered immunity (Bhattacharya et al., 2010). These plants then build up phenols at the infection site to inhibit the growth of microbial infections and prevent their spread (Chowdhary et al., 2021). These phenols are found to act as phytoalexins and nematicides against soil-borne pathogens, as they accumulate in plant tissues (Bhattacharya et al., 2010).

It is essential to note that, as a biological control agent, *Trichoderma* can induce the release of these secondary metabolites even in the absence of pathogens, preparing the plant for future infections. *Trichoderma* can induce plant resistance, including the production of phenol derivatives (Pocurull et al., 2020). This induced resistance is caused by the physiological and metabolic changes resulting from the root colonization of *Trichoderma,* which leads to the production of secondary metabolites that promote plant defense responses (Al-Hazmi & Tariq Javeed, 2016). This includes the ability of *Trichoderma* to

increase PAL activity in plants, such as tomatoes (Al-Hazmi & Tariq Javeed, 2016; Pocurull et al., 2016). Signal transduction pathways, such as those involving salicylic acid, jasmonic acid, and ethylene, trigger plant-induced resistance, as demonstrated in *Arabidopsis*, tomato, and cucumber (Hinterdobler et al., 2021; Yao et al., 2023). Once the *Trichoderma*-produced active substances activate the signal transduction pathway, this subsequently induces resistance in the plant system. The study of Nawrocka et al. (2018) demonstrated that the colonization of *Trichoderma* acted as an elicitor, inducing the systemic defense response of a plant. It also regulates the phenylpropanoid biosynthesis, and results in the *de novo* synthesis of phenols in plants. This prompts the increase of phenolic concentration suggesting that the accumulation of phenols is involved in systemic chemical response.

Although previous studies have reported that *Trichoderma* species enhance the growth of their host plants (Marra et al., 2022; Wang et al., 2020), contrary to the present findings, this does not imply that TMI harms the growth of oregano. The lack of significant differences in growth parameters between the control and TMI-treated plants at least indicates that TMI does not have any negative impacts on plant growth. Upon inoculation with TMI and the consequent upregulation of plant defense systems (as shown by the increase in phenol content), the growth of oregano was not negatively affected or inhibited. This finding aligns with previous studies on Trichoderma, which have shown that it increases the phenolic components of its host plants and their products (Nawrocka et al., 2018; Dini et al., 2020, 2021). Therefore, we can infer that the addition of *Trichoderma* Microbial Inoculant has successfully increased the plant defense systems of oregano, as manifested in the increase of phenolic compounds without negatively affecting plant growth, thus suggesting the decoupling of the growth-defense trade-off.

CONCLUSION

The current study examined the relationship between *Trichoderma* species and oregano using Biospark^{TM} *Trichoderma* Microbial Inoculant (TMI). Specifically, the growth and phenolic content responses of oregano upon inoculation with TMI were assessed through the measurement and comparison of selected morphological parameters and quantification of its total phenolic contents. This study is the first known paper in the Philippines that observed the interactions and responses of oregano with *Trichoderma*.

From the results, it has been revealed that no significant differences were found between the growth of control and TMI-treated oregano within the 49-day time frame of the study, under the conditions in which the experimentation was conducted. Many studies have started from the inoculation of seedling or seed with experiment timeline varying from 14-90 days to about 4-6 months in various plants, respectively (Banaay et al., 2012; He et al., 2020; Marra et al., 2022; Wang et al., 2020). As such, it is essential to note that the time frame used in the research, along with other considerations such as the use of plant cuttings and the small number of replicates, may have an impact on the obtained results. Despite this, it is worth noting that TMI did not harm the growth of oregano, indicating that *Trichoderma* does not impede plant growth, even when its secondary metabolites are upregulated. This is evident in the significant enhancement of the total phenolic content of the essential oil in the leaf of the oregano plant inoculated with TMI. This suggests that the upregulation of plant defense mechanisms did not compromise the plant's growth, indicating the decoupling of the defense-growth trade-off typically observed in plant responses to pathogenic organisms. Furthermore, the main phenolic components of oregano essential oil, attributed to its antioxidant and antimicrobial activity–carvacrol and thymol–were detected in both the TMI-treated and control samples. This further demonstrates the potential of oregano to enhance phenol content for use in drugs and food without compromising plant growth.

In order to obtain broader understanding on the impact of *Trichoderma* in the vegetative growth and phenolic content of *O. vulgare*, it is recommended that future studies start testing from seeds/seedlings, increase the number of replicates, use sterilized soil to isolate the effect of the inoculant, and quantify the main phenolic compounds carvacrol and thymol as well as other compounds that may have been impacted by *Trichoderma* inoculation.

STATEMENT OF AUTHORSHIP

The first author conducted the study proposal, experimentation proper, laboratory analysis, data processing, interpretation and analysis, statistical analysis, and led the manuscript writing. The second author led the calibration of laboratory processing methodologies, conceptualization and design of the study, conducted extended data analysis, and reviewed the manuscript. The third author led the review of the manuscript, conceptualization and design of the study, calibrated the methodology for experimentation proper, and conducted data analysis.

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