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Induction of systemic resistance in tomato against *Ralstonia solanacearum* using different elicitors

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Abstract

Ralstonia solanacearum that causes a vascular wilt disease and has been ranked as the second most important bacterial pathogen and it is one of the most destructive pathogens identified to date because it induces rapid and fatal wilting symptoms in host plants. Potential of thyme essential oil, tea tree essential oil and oregano essential oil in inducing systemic acquired resistance in tomato plants against *R. solanacearum* was evaluated. The study was lay-outed using CRD, replicated three times with five treatments: T1-control (negative control), T2- Salicylic acid (Positive control), T3-Tea Tree essential oil, T4- Thyme essential oil, and T5-Oregano essential oil. The experimental pots was inoculated with the bacteria and plant essential oils were applied after 2 hours of infestation, then pots were sealed with cellophane for 7 days and tomato seedlings were transplanted after 3 days of aeration. The result revealed that the different elicitors were effective in reducing disease incidence and disease severity in tomato plants. It also increase number of survival plants ranges from 55.55-77.78%, plant height increment and number of leaves. This result implies that tea tree oil, thyme essential oil and oregano essential oils are not only effective elicitors in inducing systemic resistance in tomato against *R. solanacearum* but it can also improve its growth and development.

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Introduction

The tomato (*Lycopersicum esculentum*) belongs to Solanaceae family. In the Philippines, it is a popular fruit vegetable that is widely grown as a secondary crop. It's one of the commodities that helps farmers make money. Because of its many uses, including food and skincare, the crop is thought to have a considerable market potential both locally and internationally (Department of Agriculture, 2021).

Tomato production reached 76.47 thousand metric tons in the second quarter of 2021. It increased by 3.0% compared to the 74.27 thousand metric tons produced in the same quarter of 2020 (PSA, 2021). With 28.87 thousand metric tons, Ilocos Region remained the top tomato producer, accounting for 37.8% of the country's total output this quarter. Central Luzon and Cagayan Valley came in second and third, with 10.9 percent and 9.7 percent, respectively (PSA, 2021).

However, soil-borne pathogens are a problem everywhere tomatoes are produced. One of the most devastating soil borne pathogen is *Ralstonia solanacearum* that causes a vascular wilt disease and has been ranked as the second most important bacterial pathogen and it is one of the most destructive pathogens identified to date because it induces rapid and fatal wilting symptoms in host plants (Yuliar *et al*., 2015). *R. solanacearum* is a soil- and water-borne bacterium which invades and colonizes tomato plants via root wounds; it eventually enters the xylem vessels and causes the collapse of infected tomato plants (Zhang *et al*., 2017).

In the Philippines, crop losses consistently reach 30-80% in bacterial wilt-infested fields (Miller *et al.*, 2005). The universal control methods of *R. solanacearum* is very difficult because of its species complex are so diverse. Within the Philippines, cultural practices, such as the use of mulch and reduced soil cultivation, have not been successful in reducing bacterial wilt incidence (Miller, *et al.*, 2005). The use of bacterial wilt-susceptible commercial cultivars grafted onto bacterial wilt-resistant rootstocks has been demonstrated to be an effective management tool in both the Philippines and Bangladesh (Miller *et al*., 2005). This is in conformity with the study of Manickam *et al*. (2021) that new eggplant rootstocks can be considered as alternatives to the rootstocks currently used for commercial production of tomatoes because it showed low wilting percentage at 0.0–20.0% during the hot-wet season. Enhancing host resistance with elicitors, which addresses environmental concerns, is another useful disease management technique. The perception of a pathogen or elicitors activates inducible plant defenses. Elicitors are detected by receptors that are either on the cell surface or inside the cell (Dardick and Ronald, 2006; Dipathi, *et al*., 2019). The recognition of elicitors triggers the plant's overlapping signaling responses (Kim *et al.*, 2006; Wu, *et al*., 2014). Plants respond in a variety of ways when they recognize the elicitor. In many plant-pathogen interactions, salicylic acid (SA) has been found to be a key signaling molecule involved in defense responses to pathogen attack (Shetty *et al*., 2008; Guamizo *et al*., 2020).

Plant extracts offer antibacterial properties that are effective against plant diseases. Essential plant oils contain a variety of volatile chemicals, including aliphatic aldehydes, terpenoids, esters, and alcohols, in addition to plants (Chouchan, *et al*., 2017). Fungicidal and bactericidal properties are well known in medicinal essential plant oils and their active components (Ji *et al*., 2005).

Tea Tree Oil (TTO) derived from *Melaleuca alternifolia* plant is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. Terpenes are volatile, aromatic hydrocarbons and may be considered polymers of isoprene, which has the formula C_5H_8. TTO possesses antibacterial, anti-inflammatory, antiviral, and antifungal properties. With biological activity, The antimicrobial activity of TTO is attributed mainly to terpinen-4-ol, a major component of the oil (Carson *et al*., 2006).

Thyme essential oil (TEO) derived from *Thymus* is composed of phenolic components with thymol, carvacrol, geraniol and 1,8-cineole as the major components (Ben-Jabeur *et al*., 2015; Moutassem, *et al*., 2019). Thymol had antifungal activity against pathogenic fungi and other plant diseases of several fruits and vegetables (Angelini, *et al*., 2006; Sergvic-Klaric *et al*., 2007). It has been reported that TEO was effective in inducing systemic acquired resistance in tomato against gray mold and *Fusarium* wilt (Jabeur and Hamada, 2014), *Fusarium oxysporum* f. sp. *ciceris* (Foc) and complete inhibition of *Phytophthora infestans* with a TEO concentrations of 8.0mL L-1 (Mohamedy and El-latif, 2015) and considerable anti-*R. solanacearum* (Pradhanang *et al*., 2003).

Oregano essential oil is composed of 27 chemical compounds and the most abundant bioactive components were monoterpenes and sequiterpenes. Monoterpene carvacrol was the main compound, which comprised 84.38% of the identified compounds and several other natural compounds were reported, which include p-cymene, y-terpinene, B-caryophyllene, and terpinen-4-ol 9 (Hao, *et al*., 2021). Rienth *et al*. (2019) reported that oregano vulgare essential oil vapour treatment during 24h post-infection proved to be sufficient to reduce downy mildew development by 95%. Karsou and Samara (2021) emphasized in their study that essential oils including oregano as plant resistance elicitors are promising for the loose smut disease management in barley and wheat and can be considered a novel and risk-free biocontrol agent for plant disease control, intensifying crops production under a reduced need for synthetic chemicals.

In this study, essential oils in thyme, oregano and tea tree were investigated under greenhouse conditions as elicitors in inducing systemic resistance in tomato against *Ralstonia solanacearum*.

In the Philippines, crop losses consistently reach 30 to 80% in bacterial wilt-infested fields (36). Because strains within the R. solanacea-rum species complex are so diverse, the development of universal control methods is difficult. Within the Philippines, cultural prac-tices, such as the use of mulch and reduced soil cultivation, have not been successful in reducing bacterial wilt incidence (36). The use of bacterial wilt-susceptible commercial cultivars grafted onto bacterial wilt-resistant rootstocks has been demonstrated to be an effective management tool in both the Philippines and Bangladesh (36).

Materials and methods

Experimental Design and Treatments

The study was laid out using Completely Randomized Design (CRD) with three replications and five treatments. The treatments were as follows:

T1- No application (Negative control)

T2-Salicylic Acid (700µL SA + 6.3mL of 70% Ethanol + 0.1% detergent + 56mL of water)

T3- Tea Tree Oil (700µL tea tree oil + 6.3mL of 70% Ethanol + 0.1% detergent + 56mL of water)

T4-Thyme Essential Oil (700µL thyme essential oil + 6.3mL of 70% Ethanol + 0.1% detergent + 56mL of water)

T5-Oregano Essential Oil (700µL oregano essential oil + 6.3mL of 70% Ethanol + 0.1% detergent + 56mL of water)

The solution in the essential oils was based on the study of Pradhanang, *et al*. (2003) on the different essential oils against tomato bacterial wilt in which they used 700 µL of essential oils dissolved in 6.3 mL of 70% ethanol and detergent at 0.1% in 56 mL of water for stable essential oil and were used to treat the infested soil.

Collection and Pathogenicity test of Ralstonia solanaearum (Rs)

In the field, a wilted tomato infested with Ralstonia solanacearum was collected.

The pathogenicity of the infected tomato plant was tested using Singh *et al*., method's (2018). To produce bacterial oozing, the infected plant seedling was submerged in sterile water. A 6-day-old tomato seedling was dipped in the bacterial suspension and transferred to an empty sterile test tube for 5 minutes of air exposure before being put to sterile distilled water and incubated until wilting signs appeared which took roughly 2 weeks.

Evaluation of Different Essential oils for Bacterial Wilt

The shoots infected with *Rs* were soaked in sterile distilled water and incubated for 24 hours. Growth of isolated bacteria were determined with the presence of cloudiness in water solution.

The procedure in inoculating *Rs* was based on the study of Pradhanang *et al*. (2003). A 100 mL bacterial solution was inoculated in a 900mL of soil in a polyethylene bag and thoroughly mixed. After 2 hours treatments were applied to each bag and mixed again. A plastic cover were placed at the top of the pot and plastic straw/wire were used to secure the bag to prevent escape of volatiles until 7 days and plastic cover were removed and aerated for 3 days to vent excess volatiles (Fig. 1). Then these were ready for transplanting of tomato seedlings. There were three pots per treatment with a total of 45 experimental pots.

Fig. 1. Treatment application (a); aeration of pots (b); and transplanted tomato seedlings (c).

Statistical Design and Analysis

All the experiments were laid out in Complete Randomized Design (CRD) replicated three times. The treatment means were compared using the Tukey's Honest Significant Difference (HSD) test.

Data gathered

1. Incubation period of the disease. The number of days from inoculation to the production of first visible symptoms was recorded.
2. Disease severity. The disease severity were recorded using the scale with corresponding fig.s below by Borines *et al*. (2015) and the level of resistance was based on Ghulam *et al*. (2016):

Scale	Percent coverage of Wilting	Corresponding fig.	Level of Resistance/ Susceptibility
1	No wilting		R (Resistant)

Scale	Percent coverage of Wilting	Corresponding fig.	Level of Resistance/ Susceptibility
2	Slight wilting (1-25% wilting)		MR (Moderately Resistant)
3	Moderate wilting (26-50% wilting)		MS (Moderately Susceptible)
4	Severe wilting (51-75% wilting)		S (Susceptible)
5	Almost dead/dead (>75% wilting)		HS (Highly Susceptible)

3. Percent Disease Index (PDI). The percent disease index was determined using this formula proposed by Wheeler (1969):

$$PDI = \frac{\text{Sum of the Individual disease rating}}{\text{Total number of fruits observed x Maximum grade}} \times 100$$

4. Disease Incidence. This was computed by dividing the number of infected plants to the total number of plants multiplied by 100.

5. Percent Survival. This was computed by dividing the number of plants inoculated to the total number of plants survives.

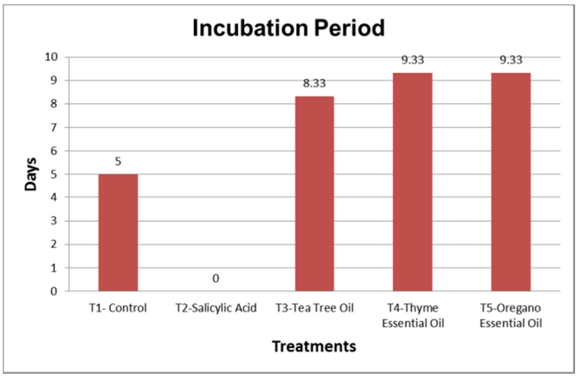
6. Number of leaves. The number of leaves was counted at the end of the experiment.

7. Plant height. The plant height was measured using a ruler/tape measure after the end of the experiment.

Results and discussions

Effect of treatments on the number of days to incubation

There was no significant variation observed between the different elicitors and the control on the days to first symptom development (Fig. 2). However, a numerical differences was observed where control was the first to develop its symptoms at 5 Days After Transplanting (DAT) the tomato seedlings followed by tea tree oil at 8.33 DAT, thyme essential oil and oregano essential oils at 9.33 DAT, respectively. As observed, salicylic acid (positive control) has no recorded infection of bacterial blight.



CV=1.53

[1]Means in a column followed by common letter/s are not significant different at 5% HSD

Fig. 2. Bacterial wilt incubation period as affected by the different elicitors.

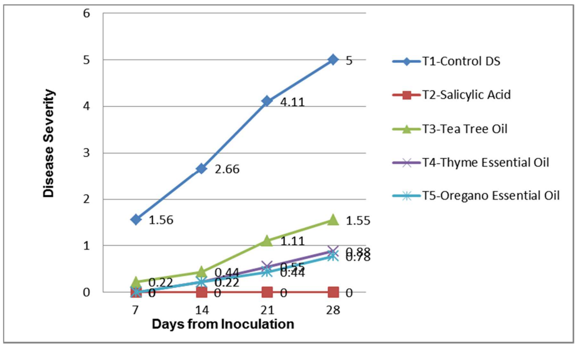


Fig. 3. Bacterial wilt disease severity ratings as affected by the different elicitors at 7, 14, 21, and 28 days after treatment.

Effects of treatments on disease incidence and severity

The results in bacterial wilt incidence revealed significant differences between treatment means at 7, 14, 21 and 28 days after inoculation (Table 1). The salicylic acid has zero infection, while plants applied with thyme essential oil and oregano essential oil has lesser incidence with the same mean of 22.22 than tea tree oil with 44.45 and is comparable to control with 100% infection of bacterial wilt (Fig. 4).

Table 1. Percentage of infected seedlings as affected by the different elicitors at 7,14,21 and 28 days after treatment.

Treatments	Disease Incidence			
	Days After Inoculation			
	7^{ns}	14*	21*	28*
T1- Control	33.33	77.78^{a}	100^{a}	100^{a}
T2-SA	0.00	0.00^{b}	0.00^{b}	0.00^{b}
T3-Tea Tree Oil	22.22	22.22^{ab}	33.33^{b}	44.45^{ab}
T4-Thyme Essential Oil	0.00	11.11^{b}	22.22^{b}	22.22^{b}
T5-Oregano Essential Oil	0.00	11.11^{b}	11.11^{b}	22.22^{b}
CV	1.84	1.57	1.31	1.10

[1]Means in a column followed by common letter/s are not significant different at 5% HSD

Analyzed data on disease severity exhibited significant differences between treatment means at 14, 21 and 28 days after inoculation (Fig. 3). Salicylic acid, oregano essential oil, thyme essential oil and tea tree oil does not differ significantly and showed less severity ratings ranges from 0-1.55 ratings compared to negative control with a rating of 5 at 28 Days After Inoculation (DAI).

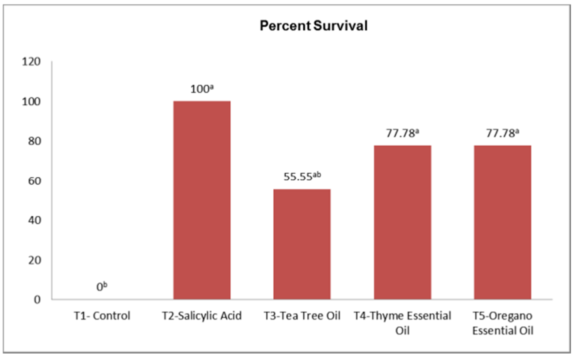
Fig. 4. Growth of tomato seedlings 28 days after inoculation.

This result implies that the different elicitors were effective in reducing disease incidence and disease severity in tomato plants. This action in the elicitors is due to induce resistance in plants. In a similar study conducted by Pradhanang *et al*. (2003) thymol were effective in reducing *R. solanacearum* population and bacterial wilt incidence of tomato grown in infested soil. This result is in conformity with the study of Ji *et al*. (2005) that thymol when applied as biofumigant significantly reduced bacterial wilt incidence. Ben-Bajeur *et al.* (2015) also reported similar results that thyme oil significantly reduced *Botrytis cineria* colonization on pre-treated detached leaves when compared to untreated leaves and displayed a significant decrease in *Fusarium* wilt severity in tomato plants. The mode of action of thyme essential oil was proxidases accumulation was the first defense activation signal which might be explained by the biological effect of essential oils and their constituents causing oxidative burst by accumulation of reactive oxygen species (ROS) (Ben-Bajeur *et al*. (2015). Rienth *et al*. (2019) also conforms to the result based on the result of their study that oregano essential oil efficiently suppressed downy mildew development in grapevines by 95 percent.

The underlying mechanism in the host-pathogen essential oil interaction shows clearly that Oregano vulgare vapour triggered a multilayered immune system of the plants and the gene expression analysis revealed a complex activation of hormonal crosstal involving Jasmonic, Ethylene, and Salicylic acid biosynthesis and their signaling cascades (Rienth *et al*., 2019). Similar result was observed in the study of Dalio *et al.* (2020) when spraying tea tree essential oil on a field of banana plants infected with *Fusarium oxysporum f. sp. cubense* and green house plants infected with *Xanthomonas campes*tris developed resistance. The effectiveness of tea tree oil is attributed to its components terpenes and their alcohols that was found to be an effective antiseptic, bactericidal (Carson, *et al*. 1993; Carson *et al*. 2006) and effective fungicide (Shao *et al*., 2013).

Effects of treatments on the percent survival of plants

The percent survival of tomato plants as affected by the different treatments exhibited significant differences between treatment means (Fig. 5). Salicylic acid (positive control) has 100% percentage survival plants, followed by Thyme and Oregano essential oils with a survival rating of 77.78% and they do not differ significantly with the positive control. This was then followed by Tea tree oil with 55.55% percentage survival which is comparable to the zero percentage survival in control/untreated.



Cv= 0.67

[1]Means in a column followed by common letter/s are not significant different at 5% HSD

Fig. 5. Percent survival of tomato seedlings as affected by the different elicitors.

This result implies that elicitors were effective in inducing systemic resistance in plants that leads to the increase percent survival in tomato plants. Based on the studies of Reinth *et al*. (2019) and Oliveira *et al.* (2017) the antimicrobial or antifungal activity of essential oils like tee-tree, oregano and thyme might mainly be caused by the properties of their terpenes/terpenoids, that due to their highly lipophilic nature and low molecular weight are capable of disrupting the cell membrane, causing cell death or inhibiting the sporulation and germination of fungi. According to Thakor and Suhal (2012) chemical elicitors affect production of phenolic compounds and activation of various defense-related enzymes in plants. In addition, Stitcher *et al*. (1997) said that once resistance or SAR is generated in plants against a given disease, it retains its efficacy for weeks, if not the entire cropping season.

Effects of treatments to plant height increment and number of leaves

The plant height increment of tomato seedlings as affected by the different elicitors exhibited significant differences between treatment means at 14, 21 and 28 days after inoculation (Table 2). The same trend was observed from 7, 14, 21 and 28 DAI where Salicylic acid (positive control) obtained the highest increment followed by thyme essential oil, oregano essential oil and tea tree oil which do not differ significantly compared with the positive control while the least increment was obtained by the negative control.

Table 2. Plant Height increment of tomato seedlings as affected by the different elicitors at 7, 14, 21, and 28 days after inoculation.

Treatments	Plant Height Increment			
	Days After Inoculation			
	7^{ns}	14*	21*	28*
T1- Control	5.13	3.7^{c}	0.00^{c}	0.00^{c}
T2-SA	6.02	14.65^{a}	19.23^{a}	6.92^{a}
T3-Tea Tree Oil	6.82	7.73^{ab}	6.19^{ab}	3.52^{ab}
T4-Thyme Essential Oil	3.82	11.86^{ab}	13.05^{ab}	6.53^{a}
T5-Oregano Essential Oil	6.36	9.20^{ab}	12.10^{ab}	2.96^{ab}
CV	40.14	53.55	82.20	73.43

[1]Means in a column followed by common letter/s are not significant different at 5% HSD

The number of tomato leaves as affected by the different elicitors exhibited significant differences between treatment means at 7, 14, 21, and 28 DAI (Table 3). Same trend was observed in every week data where elicitors obtained higher number of leaves and do not differ significantly with each other while control obtained the lowest number of leaves.

Table 3. Number of leaves in tomato seedlings as affected by the different elicitors at 7, 14, 21, and 28 days after inoculation.

Treatments	Number of leaves			
	Days After Inoculation			
	7*	14*	21*	28*
T1- Control	5.11^{b}	4.56b	0.00b	0.00b
T2-SA	8.89a	14.78a	19.77a	22.78a
T3-Tea Tree Oil	8.89a	10.44ab	12.00ab	11.99ab
T4-Thyme Essential Oil	8.44a	14.89a	19.44a	19.89a
T5-Oregano Essential Oil	8.11a	13.00a	15.00a	15.89ab
CV	20.15	41.46	63.44	69.95

[1]Means in a column followed by common letter/s are not significant different at 5% HSD

This result implies that the plant elicitors were effective in increasing the plant height and number of leaves of the plants. This result is in conformity with the study of Pradhanang *et al*. (2003) that tomato seedlings grown in inoculated soil treated with thymol or palmarosa oil produced more root and shoot tissues and were taller.

Conclusion

The essential oils of tea tree, thyme, and oregano were tested in this study as elicitors for generating systemic resistance in tomato seedlings against *R. solancearum*. The incidence and severity of disease in plants treated with various essential oils were reduced, while plant survival was increased, according to the findings. The number of leaves and plant height both increased in plants treated with essential oils. This implies that these essential oils are effective in inducing systemic resistance of tomato seedlings against bacterial wilt. The present study provides important information that tea tree essential oils, thyme essential oil and oregano essential oils can be an alternative control as elicitors of systemic resistance in tomato seedlings that is effective in reducing incidence and severity of *R. solancearum.*

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