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ANTI-BACTERIAL ACTIVITY OF ESSENTIAL OILS AGAINST POTATO PLANT PATHOGEN (BACTERIAL WILT)

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ABSTRACT

A bacterium that lives in the soil causes bacterial wilt in potato plant. Potato seed tubers can support bacterial wilt. The potato wilt bacterium mostly lives in the roots and gets into the root system when farm tools or equipment or soil pests. The purpose of this study was to assess the effectiveness of plant essential oils (EOs) against potato bacterial wilt. The essentials were extracted from *Pinus palustris*, *Ocimum basilicum*, and *Thymus vulgaris* by steam distillation method. The morphology of the isolated bacterial pathogen from potato was examined by microscopic studies. The study investigated three different concentrations of three essential plant oils (Pine, thyme and basil) under in vitro and in vivo conditions as a result of their effects on *Ralstonia solanacearum* growth and their possibility use as potato seed pieces dressing for controlling bacterial wilt disease incidence. Among the three tested concentrations, pine oil (0.25%, 0.50%, and 1%) inhibited *Ralstonia solanacearum*'s growth in vitro more effectively than thyme and basil oils. When compared to the infected, untreated control, the foliar fresh weight of treated plants and the infected, untreated control were the same, the in vivo experiment revealed that pine oil at the three concentrations significantly reduced disease incidence and severity in potato cultivars.

Keywords: Bacterial wilt, essential oils, *Pinus palustris*, *Ocimum basilicum*, *Thymus vulgaris*.

INTRODUCTION

BACTERIAL WILT

Pseudomonas solanacearum (also known as *R. solanacearum*) is responsible for the "brown rot symptoms" in potato tubers during storage, also known as bacterial wilt (BW) symptoms. It is a pathogen that spreads through seeds and soil and has a wide range of hosts around the world. Due to its numerous pathogenic strains and extensive host range, bacterial wilt is difficult to control (Salanoubat et al. 2002). Many pathogenic strains and a wide range of hosts make controlling bacterial wilt difficult (Hayward, 1991, Mahbou Somo Toukam et al., 2009). According to Lopez and Biosca (2004), the disease cannot be controlled with resistant cultivars because the host plant's resistance may vary depending on the seed- and soil-borne pathogen and the various environmental growth conditions of the host plant.

Medicinal properties of plant essential oils and their dynamic parts are therapeutic properties as having fungicidal and bactericide effects (Ji et al. 2005). Using a variety of essential plant oils and their constituents, precursory in vitro and in vivo experiments demonstrated that some leaf thyme is effective against *R. solanacearum* (Pradhanang et al. 2003). According to Oboo et al., (2014) findings, essential plant oils could be used to treat the potato tuber disease known as brown rot. He deduced that these oils have antibacterial properties that help keep *R. solanacearum*, which causes brown rot in potato tubers.

Plant essential oils anti-microbially affect plant microbes. Essential plant oils contain a number of volatile compounds in addition to plants, including aliphatic aldehydes, terpenoids, esters, and alcohols. Additionally, in an integrated disease management system, they are utilized as biofumigants. In the field, various plant compounds are chosen to control *R. solanacearum*. Worldwide research has examined the antimicrobial effects of essential plant oils on pathogens like *R. solanacearum* (Dewick 1997; Ji et al. 2007; Deberdt et al., 2012, Lucas et al., 2012, Moghaddam et al., 2014). Thymol and carvacrol are abundant in thyme essential oil. Thus, it very well may be reasonable in controlling plant sicknesses and furthermore their subsidiaries being wealthy in phenolic acids (Lee et al., 2005, Uysal et al., 2015; Roby et al., 2013, Erturk et al., 2017).

DEFENSE ENZYMES

At specific points in the host-pathogen interaction, plant disease resistance is associated with the activation of defense mechanisms that slow infection. Peroxidase (PO) and polyphenoloxidase (PPO) are two important defense enzymes that play a crucial role in the host's initial response to infection. PO activity is frequently increased by cumulative incorporation of phenolic compounds into the cell wall during incompatible plant–microbe/elicitor interactions (Adss 2013; Soares et al. 2005). The most important enzymes that plants use to protect themselves from pathogens are peroxidase and polyphenoloxidase (Qin et al., 2015). According to Foyer and Noctor (2003), polyphenoloxidase (PPO) and peroxidase (PO) are distinct antioxidant enzymes that play significant roles in defense against membrane lipid peroxidation and oxidative

stress during pathogen infestation.

ESSENTIAL OILS USED IN THIS STUDY

Several essential oils derived from the genus Pinus (Pinaceae) are commercially important for use in anti-microbial activity, including Scots pine (*Pinus sylvestris L.*), black pine (*Pinus nigra*), and jack pine (*Pinus bansksiana Lamb.*). Numerous members of the genus Pinus (Pinaceae) are utilized in traditional medicine in their native ranges and white pine (*Pinus strobus L.*). The industrial fractional distillation of turpentine yields pinene, the most abundant terpene found in nature. α-Pinene can be found in the oil of *Pinus palustris* at concentrations of up to 65%.

Researchers have recently focused on essential oils to encompass many aspects of their contribution to the control of disease (Raveau et al., 2020) which are potential sources of microbiocide compounds. Due to the fact that they are an abundant supply of bioactive chemicals (Raveau et al., 2020). These synthetics are biodegradable to nontoxic items and are possibly appropriate for incorporated use. There are more than 300 species of herbaceous perennials and sub-shrubs in the *Thymus* genus, which is in the Lamiaceae family. In Tunisia, this family is for the most part addressed by *Thymus capitatus* Hoff. and Link. =Rchb.f., *Coridothymus capitatus* (L.) *Thymbra capitata* (L.) Cav., *Satureja capitata* L., and *Thymus vulgaris.* The antimicrobial properties of thyme essential oils have been reported (Magi et al., 2015), the majority of which are mediated by carvacrol and thymol, the oil's primary phenolic components.

In clinical and horticultural fields, basil separate has been accounted for to repress the development of contagious microorganisms, in particular *Enterococcus sp., Listeria species, Staphylococcus aureus, Aspergillus sp., Fusarium sp., Escherichia coli,* and many pathogens (Bansod et al., 2008; Bhardwaj, 2012; Carović-Stanko et al., 2010; Dambolena et al., 2010; Kocic-Tanackov et al., 2011; Piyo et al., 2009; Kumar et al., 2010).

Basil's antimicrobial compounds are to blame for this. Colpas et al., (2009) claim that an aqueous extract of *Ocimum gratissimum* caused soybean cotyledons and sorghum mesocotyls to produce phytoalexins. It also caused cucumber to become systemically resistant to *Colletotrichum lagenarium,* as evidenced by a decrease in disease incidence and an increase in chitinase production. In addition, past review announced that watery concentrate of *O. basilicum* fundamentally decreased the early curse frequency on tomato, brought about by *Alternaria solani,* under nursery and field condition (Nashwa et al, 2012). In addition, the vapour phase method revealed that the essential oil of *O. basilicum* completely inhibited the growth of the post-harvest fungal pathogen *Rhizopus stolonifer*. Another study was conducted to evaluate the effectiveness of sweet basil aqueous extract against *Sclerotium rolfsii* in-vitro and damping-off on tomato seedlings caused by *Sclerotium rolfsii* in greenhouse conditions in relation to its potent use in disease control.

PLANT PATHOGENS

Worldwide, plant pathogens result in significant crop losses. Typically, synthetic antibiotics are used to control diseases. Despite their effectiveness, their continued use caused disease outbreaks, adverse effects on the environment and plants, and frequently resulted in pathogen resistance. The need for the active search for bioactive molecules

in plants, insects, and microorganisms that could represent an alternative to chemical disease control has been prompted by the decreasing efficacy of synthetic antibiotics and the growing concern regarding their adverse effects. For sure, 'regular' metabolites may be more biodegradable than engineered ones. One of the most effective options for protecting the environment in the modern era is using natural products as plant elicitors. By altering the host plant's physical and physiological state, they can either directly or only after a pathogen challenge has occurred activate defense mechanisms (Walters and Boyle, 2005).

ANTI-MICROBIAL ACTIVITY OF ESSENTIAL OILS

Essential oils (likewise called volatile oils) are sweet-smelling sleek fluids got from plant materials (buds, fruits, flowers, barks, seeds, leaves, twigs, wood, spices, products of the soil). We are aware of approximately 3000 essential oils, of which 300 are significant to the fragrance industry. Essential oils are intricate mixtures made up of numerous compounds (Dhifi et al., 2016). Synthetically, they are gotten from terpenes and their oxygenated compounds. These oils have anti-microbial effects because of each of them. It has been demonstrated that essential oils have antibacterial, antiviral, insecticidal, and antioxidant properties. A few oils have likewise been utilized in malignant growth treatment. They have been utilized as food additives, for fragrance based treatment and in the aroma industry. Some essential oils have been shown to have potential antimicrobial properties after being tested for antimicrobial activity in vivo and in vitro (Parham et al., 2020). They appear to focus primarily on the cell membrane, disrupting its structure and resulting in cell leakage and death; secondary actions may include blocking membrane synthesis. as well as a halt to cellular respiration. Because of their high volatility and lipophilicity, the essential oils are able to easily enter the cell membrane and exert their biological effect (Chouhan et al., 2017).

REVIEW OF LITERATURE

BACTERIAL WILT DISEASE

One of the most serious diseases that affect tomatoes and other solanaceous plants is bacterial wilt. It is known that the disease affects some temperate and wet tropics as well as some subtropics. The bacterium *Ralstonia solanacearum*, formerly known as *Pseudomonas solanacearum*, is responsible for the disease. It is one of the most harming plant microorganisms (Peeters et al., 2013). Over 200 plant species in over 50 families worldwide are affected by this pathogen's strains, including ornamentals, crop plants, and weeds. The strains of *R. solanacearum* have ordinarily been named races and biovars. Race 1 or race 3 of *R. solanacearum* is responsible for potato bacterial wilt, and race 2 is rare. Race 1 is endemic to the United States and has the potential to cause bacterial wilt on a number of important crops, including tomato, potato, pepper, tobacco, and eggplant (Huerta, et al., 2015). Although infected geranium cuttings from offshore production sites have brought race 3 into the United States on multiple occasions, this race has been eradicated and is not considered established in North America. However, *R. solanacearum* race 3 biovar 2 is considered a serious threat to the potato industry in the United States due to the risk of its reintroduction and its potential to affect potato in the northern United States. The Agricultural Bioterrorism

Act of 2002 lists it as a Select Agent plant pathogen because it is important for quarantine (Lindgren et al., 2005).

SIGNS & SYMPTOMS

The signs and symptoms of potato bacterial wilt disease was depicted in Figure No.1. At the beginning phases of sickness, the primary noticeable side effects of bacterial shrink are normally seen on the foliage of plants. These side effects comprise of withering of the most young leaves at the finishes of the branches during the most sizzling piece of the day (Tans-Kersten et al., 2001). One or two leaflets may wilt at this point, and plants may appear to recover at night when temperatures are lower. Even though dried leaves remain green, the entire plant may quickly wilt and desiccate as the disease progresses under favorable conditions, resulting in general wilting and yellowing of the foliage and eventual plant death. Stunting of plants is another common symptom that can be caused by bacterial wilt in the field. These signs can happen at any point in the growth of the plant, but in the field, it's common for plants that look healthy to suddenly wilt when the fruits are growing quickly (Shen et al., 2020).

In young potato stems, contaminated vascular packs might become apparent as lengthy, restricted, dim earthy colored streaks. In youthful, delicious plants of exceptionally helpless assortments, breakdown of the stem may likewise be noticed. High temperatures (85-95 degrees Fahrenheit) favor the expression of symptoms, and the disease's symptoms may progress rapidly after infection. However, plants that don't show symptoms may remain infected latently for a long time under favorable conditions. The pathogen may survive and spread from the infected plant after infection. A sticky, milky-white exudate, which indicates the presence of dense masses of bacterial cells in infected vascular bundles, particularly in the xylem, which is responsible for transporting raw sap (water and nutrients) from roots to aerial parts of the plant, is a common sign of tomato bacterial wilt. *R. solanacearum* is a pathogen that only invades the xylem (Shen et al., 2020).

CAUSATIVE ORGANISM

Ralstonia solanacearum is a strictly aerobic, rod-shaped Gram-negative bacterium measuring 0.5-0.7 x 1.5-2.0 m in size. It is extremely sensitive to desiccation and is inhibited in culture by sodium chloride (NaCl) at low concentrations (2%). For most strains, the ideal temperature is 82-90 ºF. However, the optimal temperature for some strains is 80.5 degrees Fahrenheit (Bindal et al., 2019). When cultivating the bacterium, both liquid and solid (agar) growth media are frequently utilized. After 36 to 48 hours of growth at 82.4°F on solid agar medium, individual bacterial colonies are typically

visible. There are two primary morphologically distinct colony types: normal or virulent white or cream-colored, irregularly rounded, fluid, and opaque colonies; and uniformly round, smaller, and butyrous (dry) colonies of the mutant or non-virulent type. In liquid media, this transition from virulent to non-virulent bacterial cells occurs during storage or when oxygen is stressed. To distinguish between the two types of colonies, a TZC medium was created in which virulent colonies appear white with pink centers and non-virulent colonies appear dark red. A semi-specific medium, called changed SMSA medium, has been produced for location of *R. solanacearum* in water

and soil tests, and in plant extricates. After two to five days of incubation at 82.4 ºF, typical bacterial colonies on this medium become fluid, irregular in shape, and white with pink centers. A milky (mucilaginous) exudate that indicates the presence of bacterial cells can also be observed in freshly cut sections of infected tubers. Bacterial exudate may also be visible in the eyes or at the point where the stolon connects to the tuber as the infection progresses. It's possible that these signs or symptoms won't show up until later in the disease's progression (Hossain et al., 2021; Fujiwara et al., 2011).

BACTERIAL WILT DISEASE IN CROPS

Crops Potato (*Solanum tuberosum*); tomato (*Lycopersicum esculentum*); soybean (*Glycine max*); eggplant/aubergine (*Solanum melongena*); banana, (*Musa spp*); geranium (*Pelargonium spp.*); ginger (*Zingiber officinale*); tobacco (*Nicotiana tabacum*); bell pepper/sweet pepper (*Capsicum spp.*); olive (*Olea europea*) (Fujiwara et al., 2011).

SURVIVAL

Ralstonia solanacearum is able to spend the winter in diseased or decaying plants, wild hosts, seeds, or vegetative propagative organs (also known as other germplasm), such as tubers. Bacteria can survive for up to 40 years in water at 20–25 °C (68–77 °F) in pure water, but their population decreases in extreme conditions (temperature, pH, salts, etc.). For several years, infected land may not be usable again for susceptible crops. *R. solanacearum* can also survive in cool weather and reach a point where it is viable but cannot be cultivated. Because the bacteria usually become avirulent after recovery, this stage rarely poses a threat to agriculture (Hossain et al., 2021; Fujiwara et al., 2011).

SPREADING ROUTE OF BACTERIAL WILT DISEASE

Ralstonia solanacearum spreads via a variety of routes and causes wilting in large populations (10^8-10^{10} CFU/g tissue). The numerous strains of *R. solanacearum* can shed from the roots of both plants with and without symptoms. In addition, this pathogen can be spread by contaminated flood water, irrigation, contaminated tools, or infected seeds, as well as by bacterial ooze (which is typically used as a sign for detection) on plant surfaces. In addition, this pathogen can enter the soil or water surrounding the plant, contaminating farming equipment, or being acquired by insect vectors. The pathogen has established itself in solanaceous weeds that grow in slow-moving rivers in northern Europe. The pathogen enters the process of producing potatoes when such contaminated water is used to irrigate them. The race 3 biovar 2 strain is able to thrive in perennial nightshades, which serve as secondary hosts, and it can also cause bacterial wilt of tomato. Several nations in the European Union and the Middle East have not yet been able to completely eradicate this pathogen (Hossain et al., 2021; Fujiwara et al., 2011).

Typically, *Ralstonia solanacearum* enters the plant through a wound. *R. solanacearum* may enter the plant through both natural wounds (like those caused by flower abscission and the formation of lateral roots) and unnatural wounds (like those caused by agricultural practices or by nematodes and xylem-feeding insects). Chemotaxic attraction to root exudates and flagellar-mediated swimming motility help the bacteria get into the wounds. *R. solanacearum*, in contrast to many phytopathogenic bacteria,

may only require one entry point to cause bacterial wilt, a systemic infection (Hossain et al., 2021).

Before bacterial wilt symptoms appear, *R. solanacearum* multiplies and spreads throughout the plant after invading a susceptible host. Withering ought to be considered as the most apparent secondary effect that typically happens after broad colonization of the microbe. Tyloses can form to hinder the axial migration of bacteria within the plant when the pathogen enters the xylem through natural openings or wounds. This may occur slowly and infrequently in susceptible plants to prevent pathogen migration, but it may instead result in vascular dysfunction by unspecifically obstructing uncolonized vessels (Hossain et al., 2021).

can transmit the disease through progeny tubers, even if they don't show any symptoms, especially in cooler climates. Also, farmers not recommending some of these varieties, like "Cruza" in Rwanda, or they have problems like being sensitive to temperature and high glycoalkaloid content.

AIM & OBJECTIVES OF THE PROJECT

AIM

To evaluate the antibacterial effects of three essential plant oils on the growth of *R. solanacearum* in vitro and in vivo conditions.

OBJECTIVES

The study had the following objectives:

(i) Evaluate the antibacterial effects of three essential plant oils on the growth of *R. solanacearum* in vitro.

(ii) Improving the efficacy of application methods and controlling *R. solanacearum* in vivo to reduce the frequency and severity of potato wilt.

(iii) Examining the effect of essential plant oils on the vegetative growth of potato plants and the activity of enzymes and phenols in treated potato plants.

MATERIALS AND METHODOLOGY

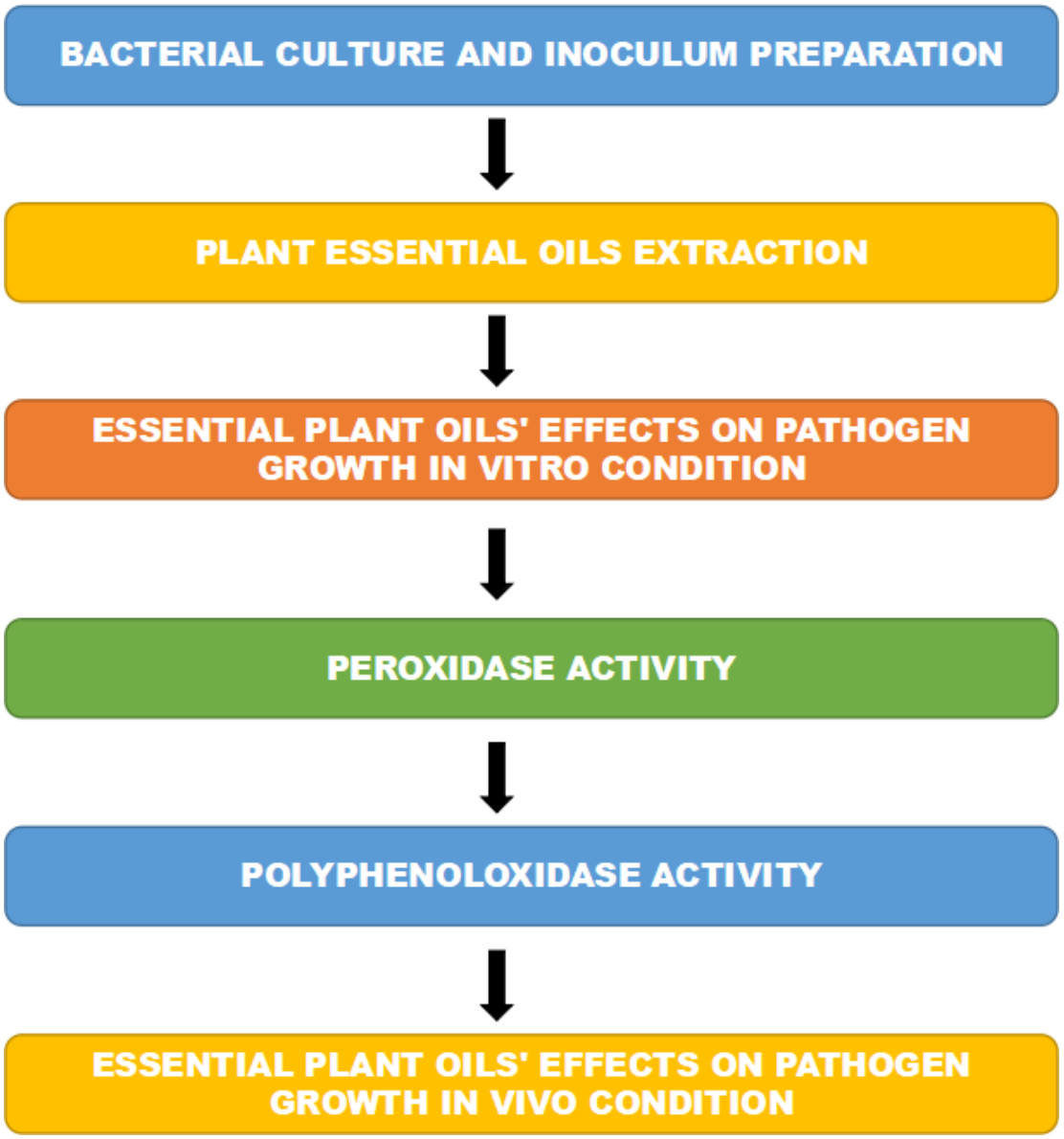

Figure No.1: Flowchart of the work

BACTERIAL CULTURE AND INOCULUM PREPARATION

The bacterial isolate used in this study were purchased from Biodeavour Research Lab, Porur, Chennai that isolated from infected potato tubers. A well-characterized *Ralstonia species* were characterized and confirmed by microscopic study. On Nutrient agar medium, the pathogenic bacterial isolate were grown for 48 hours at 28 °C. Based on their morphology, the tested bacterial isolates' colonies were harvested, suspended in nutrient broth, and grown for three days at 28 °C. The cultures were centrifuged for 10 minutes at 3500 rpm. Bacterial pellets were suspended in distilled water (Abd-Elrahim et al., 2015; Kelman, A. 1954).

PLANT ESSENTIAL OILS EXTRACTION

The steam distillation method produced highly purified essential plant oils such as Pine oil (*Pinus palustris*), Basil oil (*Ocimum basilicum*), and Thyme oil (*Thymus vulgaris*).

Based on the findings of a previous study (Pradhanang et al., 2003), three concentrations (0.25, 0.50, and 1% vol/vol) were prepared from each original oil and tested on species of *Ralstonia*. To make the tested essential plant oils more soluble in water, they were emulsified in Tween 80 (1:1). The required quantity of oil was combined with twenty milliliters of water to achieve final concentrations of 0.25 percent v/v, 0.50 percent v/v, and 1 percent v/v, respectively (Pradhanang et al., 2003; Abd-Elrahim et al., 2022).

ESSENTIAL PLANT OILS' EFFECTS ON PATHOGEN GROWTH IN VITRO CONDITION

The research was conducted at the Biodeavour Research Lab in Porur, Chennai, Tamil Nadu. On Nutrient agar plates, the antagonistic effect of essential plant oils on the growth of *Ralstonia species* was investigated. Using a sterilized L-shaped rod spreader, 100 microliters of *Ralstonia species* suspension were spread on the surface of the plate. Then a 6 mm diameter of disc (into each disc the respective essential oils was put. After 48 hours, the inhibition zone around the well was measured in millimeters (Abd-Elrahim et al., 2022).

PEROXIDASE ACTIVITY

Fresh samples of 1 g of potato leaves taken from the infected plants 45 days after planting were used to measure the enzymes. Kochba et al. (1977) provided the formula for calculating peroxidase. The enzyme was measured in mg/g of fresh weight and its color density was read using a spectrophotometer 601 at a wavelength of 425 nm.

POLYPHENOLOXIDASE ACTIVITY

Fresh samples of 1 g of potato leaves taken from the infected plants 45 days after planting were used to measure the enzymes. Lisker et al. (1983) provided the formula for calculating polyphenoloxidase. The enzyme was measured in mg/g of fresh weight and its color density was read using a spectrophotometer 601 at a wavelength of 495 nm.

ESSENTIAL PLANT OILS' EFFECTS ON PATHOGEN GROWTH IN VIVO CONDITION

The potato cultivars' uniform tubers were purchased from nearby nursery gardens. With one tuber per pot, potato seed pieces were planted in plastic sterilized 25 cm pots filled with 10 kg of *Ralstonia species*-free sandy-clay soil (1/1, v/v) from pest-free fields in nursery gardens. *Ralstonia species* isolate suspensions, previously propagated in liquid culture and grown for three days at 28 °C with 50 ml/kg soil, were used for the soil infestation. The essential oil which proved to be effective in vitro experiment, were used for the in-vivo study. To ensure that the buds were not damaged, tubers were separately soaked in 250 milliliters of each essential plant oil concentration for 15 minutes before being planted in pots with infested soil. Only concentrations of tested essential plant oils were used to treat control treatment pots, which were maintained in the same conditions without infestation. Five pots were utilized for every specific treatment (Winstead, N. N. 1952; Abd-Elrahim et al., 2022).

After 45 days of planting, the percentage of wilted plants was used to determine the disease's incidence. Using the modified scale provided by Winstead and Kelman (1952), disease severity was assessed 45 days after the first onset of disease:

The following equation was used to determine the severity of the disease:

$$\mathbf{Diseaseseverity\%} = \frac{\sum(\mathbf{No.\,ofwiltedplantsineachcategory} \times \mathbf{wiltgrade})}{\mathbf{TotalNo.\,ofplants} \times \mathbf{highestgrade}} \times 100$$

The following formula was used to determine the percentage of disease reduction incidence and severity:

$$\mathbf{PercentReduction} = \frac{\mathbf{C} - \mathbf{T}}{\mathbf{C}}\mathbf{X}100$$

The weight of the tubers (in grams) and the fresh weight of the plant (in grams) were determined 50 days after planting, while the enzyme activity was determined 35 days after planting.

RESULTS & DISCUSSION

BACTERIAL CULTURE AND INOCULUM PREPARATION

After late blight caused by Phytophthora infestans, bacterial wilt, or brown rot disease, caused by *Ralstonia solanacearum* (Smith) (Figure 3), is the most serious potato disease in subtropical and tropical regions, as well as in some cool temperate regions of the world. It is the most serious potato disease in Africa, primarily affecting Uganda, Rwanda, Ethiopia, Kenya, Burundi, Nigeria, Madagascar, and Cameroon in the central and southern regions. Potato exports to European markets were restricted as a result of its tuber infection (Uwamahoro et al., 2018)

Due to the fact that there are no chemicals known to effectively control bacterial wilt, its management with chemicals appears nearly impossible or more complicated, making it the most problematic disease. Alongside the need of compelling synthetics, pesticide protections as well as negative impacts of synthetic compounds on shopper wellbeing and regular foes limit their utilization at worldwide level (Muthoni et al., 2013; Strange and Scott, 2005; Priou et al., 2001; Hammes, 2013; Wagura et al., 2011; Masengesho et al., 2012; Guchi, 2015).

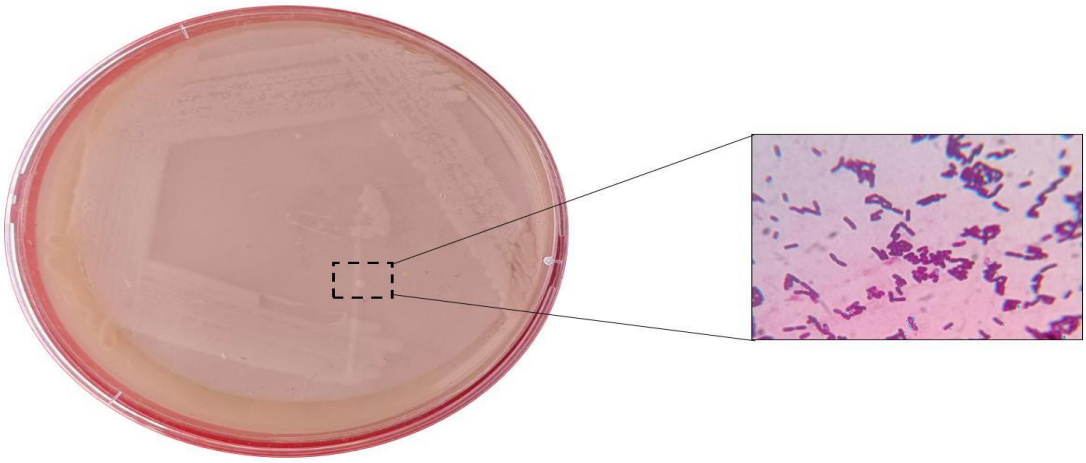

Figure No.2: Culture morphology and gram staining image of *R. solanacearum*

PLANT ESSENTIAL OILS

Table 1 displays the data's essential oil extraction rate following steam distillation

treatment. Each experiment yielded a mass of 100–200 g of raw material (m1) and a mass of about 0.5 g of essential oil (m2).

Table No.1: Experimental results of extraction of essential oils by steam distillation.

Raw Material Used	m1 (gram)	m2 (gram)
Pinus palustris	189.13	0.50
Ocimum basilicum	110.61	0.46
Thymus vu lgaris	121.80	0.18

ESSENTIAL PLANT OILS EFFECTS ON PATHOGEN GROWTH IN VITRO CONDITION

The data in Table 2 and Figure 4 make it abundantly clear that *Ralstonia solanacearum* growth on the agar plate is inhibited by all of the utilized essential plant oils at varying concentrations. The results showed that the three tested concentrations of pine oil were most effective at inhibiting the growth of Ralstonia solanacearum isolates, followed by thyme and basil oils (0.25, 0.50, and 1% vol/vol). Figure 4, 5, and 6 depicts the outcomes of the antibacterial activity of all essential oils.

In our study, we were found to be the most effective inhibitors of *R. solanacearum* growth, the three essential plant oils that were subjected to in vitro testing were chosen. It is important to note that the *R. solanacearum* isolate was effectively inhibited from growing by using pine oil at various concentrations (Figure 4). Additionally, the essential oils of thyme and basil have a limited inhibitory effect on *R. solanacearum* growth. As a result, the in vivo study did not include basil oil or thyme oil. It was discovered that the phenolic components of the thyme essential oils are primarily responsible for the oils' antibacterial properties (Figure 7). Thymol and carvacrol, two phenols, are the compounds with the greatest quantitative significance. According to Sivropoulou et al., (1996) these two phenolic compounds have strong antimicrobial properties. Aligiannis et al. (2001). The hydrocarbons γ-terpinene and p-cymene are two examples of monoterpenes that are abundant in thyme.

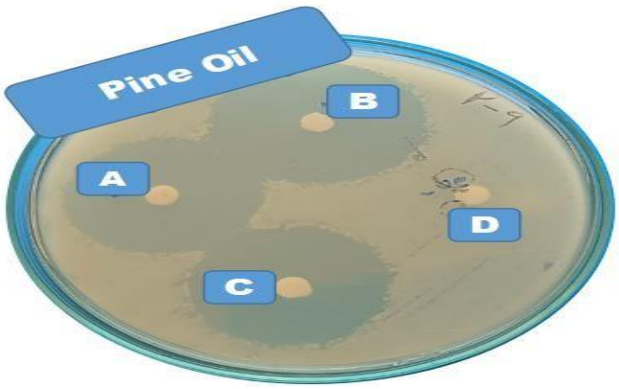

Figure No.3: Anti-bacterial Activity of Pine Essential Oils against *R. solanacearum* isolate. A) 0.25 %, B) 0.50 %, C) 1 % and D) Negative Control (Distilled water).

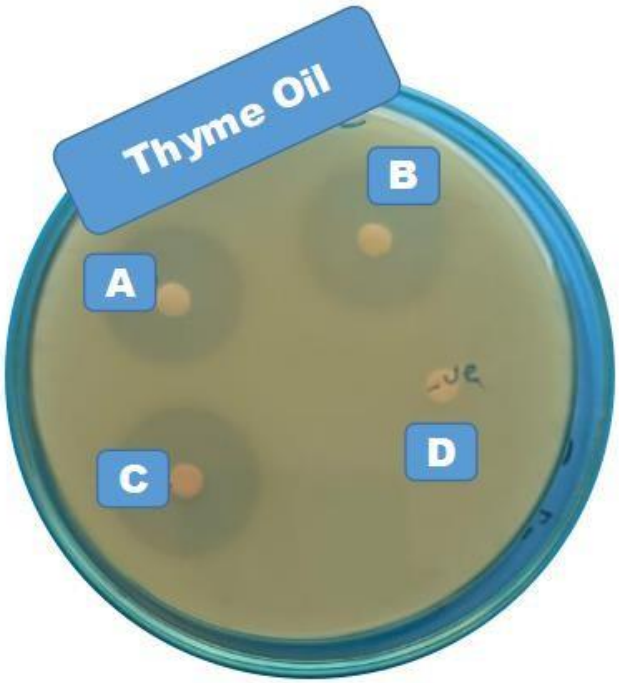

Figure No.4: Anti-bacterial Activity of Thyme Essential Oil against *R. solanacearum* isolate. A) 0.25 %, B) 0.50 %, C) 1 % and D) Negative Control (Distilled water).

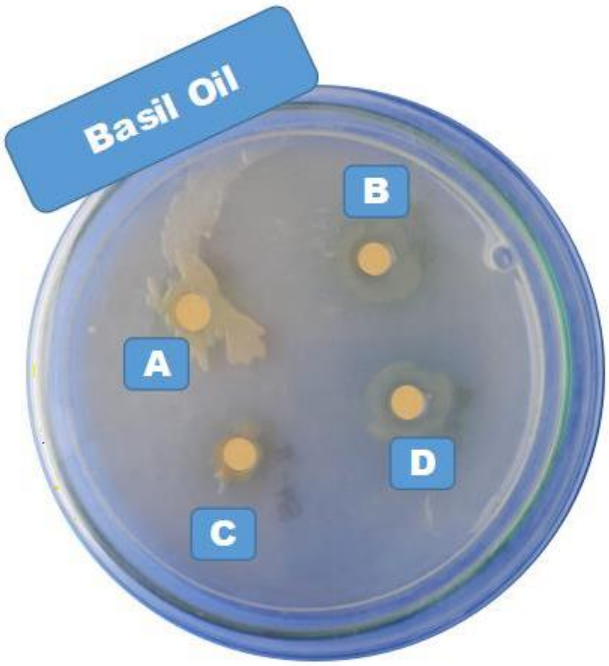

Figure No.5: Anti-bacterial Activity of Basil Essential Oil against *R. solanacearum* isolate. A) 0.25 %, B) 0.50 %, C) 1 % and D) Negative Control (Distilled water).

Table No.2: Anti-bacterial Activity of Varied Concentration of Essential Oils against *R. solanacearum* isolate

Essential Oils	Zone of Inhibition in mm			
	0.25 %	0.50 %	1 %	- Ve
Pine Oil	22	28	34	-
Basil Oil	12	14	19	-
Thyme Oil	1	8	11	-

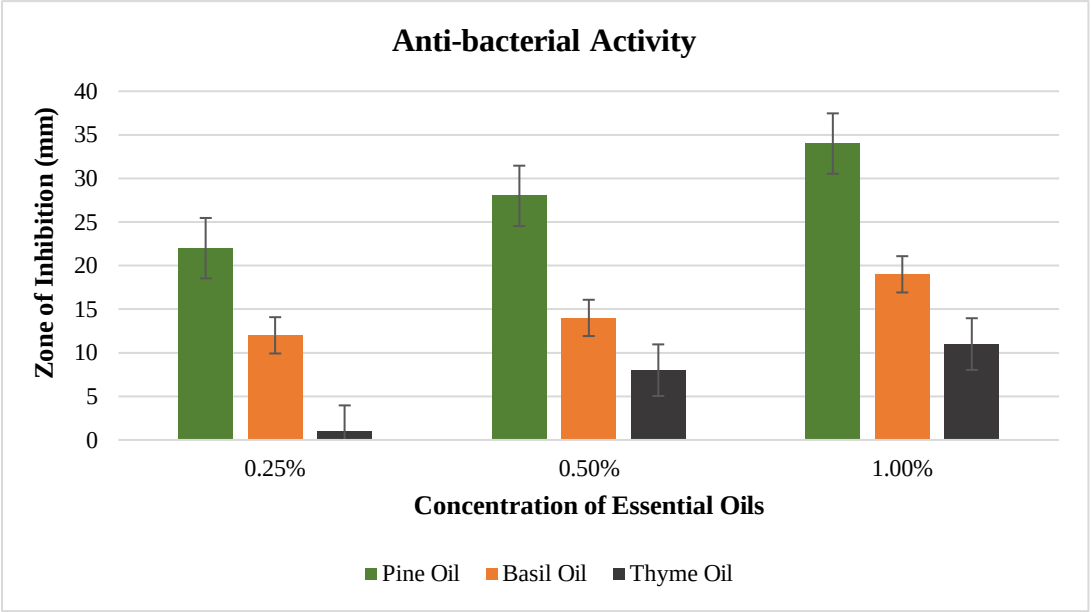

Figure No.6: Anti-bacterial activity of essential oils with varied concentration

Likewise, action of antibacterial movement of fundamental plant oils were reported by (Ultee et al., 1999, Dorman and Deans, 2000, Rota et al., 2008). Through the hydrophobic components of carvacrol, eugenol, eugenol acetate, and caryophyllene, which react with the cell membranes of the different bacteria and alter the permeability for H + and K + cations, they discovered that clove essential oil can harm several essential bacterial cell processes, such as the change in DNA synthesis, the waste of turbidity, the inhibition of enzyme activity, and the decrease in metabolic processes.

PEROXIDASE ACTIVITY

Figure 8 data showed that pine, basil, and thyme at concentrations of 0.25, 0.50, and 1% provided the essential oil-treated potato plants with the highest peroxidase activity values. The highest activity values of 0.58, 0.78, and 0.9 was observed in pine essential oil. While the lowest activity values 0.45, 0.56, and 0.7 for peroxidase was observed in

basil essential oil, and 0.48, 0.58, and 0.79 for thyme essential oil. The enzymatic activity of peroxidase data was depicted in Table 3 and Figure 8.

Table No.3: Enzymatic activity of peroxidase in essential oils treated plants

Essentials Oils	**Peroxidase Activity in mg/g of plant fresh weight**		
	0.25 %	**0.50%**	**1 %**
Pine Oil	0.58	0.78	0.9
Basil Oil	0.45	0.56	0.7
Thyme Oil	0.48	0.58	0.79

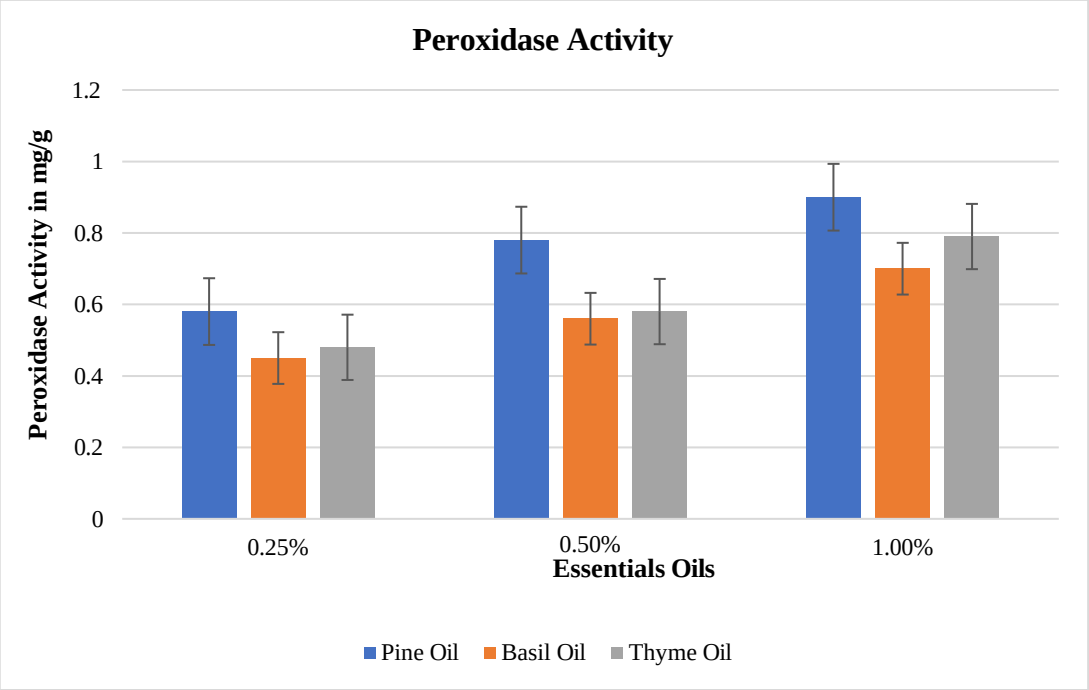

Figure No.7: Peroxidase Activity in mg/g of plant fresh weight

POLYPHENOLOXIDASE ACTIVITY

Figure data showed that pine, basil, and thyme at concentrations of 0.25, 0.50, and 1% provided the essential oil-treated potato plants with the highest polyphenoloxidase activity values. The highest activity values of 0.59, 0.68, 0.80 was observed in pine essential oil. While the lowest activity values 0.35, 0.46, and 0.72 for polyphenoloxidase was observed in basil essential oil, and 0.4, 0.51, 0.78 for thyme essential oil. The enzymatic activity of polyphenoloxidase data was depicted in Table 4 and Figure 9.

Table No.4: Enzymatic activity of polyphenoloxidase in essential oils treated plants

Essentials Oils	Polyphenoloxidase Activity in mg/g of plant fresh weight		
	0.25 %	0.50 %	1 %
Pine Oil	0.59	0.68	0.80
Basil Oil	0.35	0.46	0.72
Thyme Oil	0.4	0.51	0.78

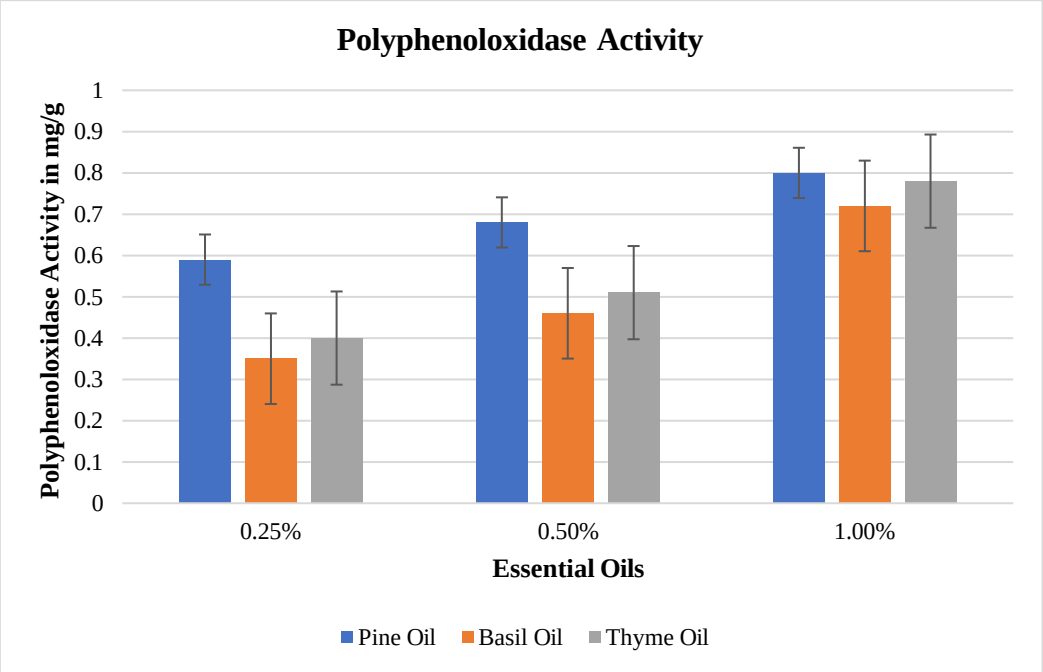

Figure No.8: Polyphenoloxidase Activity in mg/g of plant fresh weight

ESSENTIAL PLANT OILS EFFECTS ON PATHOGEN GROWTH IN VIVO CONDITION

The management of *Ralstonia solanacearum*, which caused the potato bacterial wilt disease, is currently extremely difficult due to the lack of the most efficient chemical control treatments for sanitizing *Ralstonia solanacearum*-infected fields (Figure 10). The genetic program to introduce resistant cultivars is the most reliable method for controlling *R. solanacearum* (Lebeau et al., 2011), but due to the importation of all potato seed pieces, this strategy is unavailable in many countries. These outcomes showed that pine plant oil able to inhibit the growth of *R. solanacearum* in potato plant and their impact depending on the concentration of essential oils utilized. According to our findings, varying concentrations of the essential plant oil of pine slowed the in vivo growth of *R. solanacearum.*

Figure No.9: In-Vivo Inhibition Study of *R. solanacearum* growth by Pine essential oil.

The mechanism by which essential plant oils inhibit the growth of pathogenic bacteria. Classifying the essential oils' contents, as well as their effects on the growth of pathogens and their roles in the metabolism of plants following application, was necessary for disease control. In this regard, a number of studies were conducted to explain the oil, pathogen, host plant, and their interaction-dependent mode of action of these essential plant oils. Rota et al., (2008) and Hindumathy, (2011) reported on some of these studies and they found that the inhibiting effect of plant essential oils on *R. solanacearum* development can be appended to dynamic antibacterial compounds include: steroids, terpenoids, alkaloids, geraniol, citral, flavonoids, eugenol, cytronolal, geranyl acetic acid derivation, beta cariofiln, tannins, phenolic compounds (thymol), terpene hydrocarbons (γ-terpinene) saponin, farnsul, and p-Cymene, individually. Pine oil was found to be most effective against *R. solanacearum* in this study. This suggests that pine oil's phenolic content is to responsible for its antibacterial properties, and numerous phytochemical studies have shown that pine contains phenolic compounds in significant quantities.

CONCLUSION

The purpose of this study was to determine the efficiency of three available essential plant oils (Pine, Basil, and Thyme) could be used in vivo to control potato bacterial wilt by inhibiting the growth of pathogenic *R. solanacearum* isolate. The bacterium *Ralstonia solanacearum* was inhibited most severely by the pine essential oils (1%). The fact that the essential oils of pine and thyme were found to have a greater inhibitory effect than the essential oils of basil leads one to consider using them instead of a sufficient biological control method against *Ralstonia solanacearum* in potato cultivars. However, it is essential to point out that phytotoxicity studies should be conducted to determine whether or not these concentrations hinder the plant's proper development throughout the phenological cycle. From this study, it is concluded that pine essential oil have higher potential against bacterial wilt of potatoes followed by thyme essential oil. Furthermore, minimum inhibitory concentration (MICs) is lower in pine essential oil than thyme and basil essential oil. Thus, pine essential oil has stronger anti-bacterial properties in both in-vitro and in-vivo experiments and is the most recommended in

management of *R. solanacearum*. Moreover, all the tested concentration of pine essential oil are effective in growth inhibition of *R. solanacearum* of potato under in vitro conditions. The use of the botanicals which are locally available, economically affordable, easy to prepare, nontoxic to non-target organisms and also environmentally friendly is a good management strategy for the potato bacterial wilt. The active ingredients of bioactive essential oil from Pine plant should also be identified as well as their modes of action against *R. solanacearum*

In conclusion, the integrated control of bacteria wilt in-vitro and in-vivo conditions has consistently been effective with pine oil. Despite the fact that additional greenhouse and field studies should be carried out to confirm the efficacy of essential oils in natural conditions and the dosage required to achieve a reasonable degree of disease biocontrol efficiency and economic benefits to farmers.

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