



Faculty of Agriculture, University of Poonch Rawalakot



Check for updates

# Jammu Kashmir Journal of Agriculture

ISSN: 2958-3756 (Online), 2958-3748 (Print)

<https://jkjagri.com/index.php/journal>

## ECO-FRIENDLY MANAGEMENT OF *ERWINIA AMYLOVORA* CAUSING FIRE BLIGHT OF APPLE THROUGH ESSENTIAL OILS UNDER INVITRO CONDITION

<sup>a</sup>Shah Mohammad, <sup>a</sup>Muhammad Basharat, <sup>b</sup>Fazal Haq, <sup>c</sup>Siraj Ahmed, <sup>d</sup>Suleman Khan, <sup>e</sup>Sumbel Musa, <sup>a</sup>Zobia Jabeen, <sup>a</sup>Muhammad Waris\*

<sup>a</sup> Department of Plant Pathology, Balochistan Agriculture College Quetta, Pakistan.

<sup>b</sup> Directorate of Post-Harvest and Food Technology, ARI, Sariab Quetta, Pakistan.

<sup>c</sup> Department of Agronomy, Balochistan Agriculture College Quetta, Pakistan.

<sup>d</sup> Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan.

<sup>e</sup> Deputy Director Office, Agriculture Extension Department Quetta, Pakistan.

### ABSTRACT

The apple (*Malus domestica*) belongs to the genus *Malus*. Family *Rosaceae*. It is grown worldwide and especially temperate region of Balochistan, Pakistan. Apple is affected by many diseases from one of the most damaging diseases is Fire blight of apple throughout the world. The research was conducted at Balochistan Agriculture College Quetta to observed the incidence of disease and management through essential oil *invitro* condition. Survey was accomplished to checked incidence of fire blight of apple at various districts of Balochistan. The disease incidence percent ranged between 12.33-18.75% at different locations. The maximum incidence 18.75% was recorded at Mastung. Followed by Quetta 13.33%, Pishin 12.50%, and Killa Abdullah 16.43%. Five essential oils namely *Syzygium aromaticum* (clove oil), *Cinnamomum verum* (cinemon oil), *Cocos nucifera* (coconut oil), *Rosa indica* (Rose oil), and *Sesamum indicum* (sesame oil) were tested for their antibacterial efficacy against the pathogen. Screening was performed *In-vitro* on agar plates inoculated with *E. amylovora*. Complete randomize design was used for experiment and data was statistically analyzed. It was revealed that all oil made inhibition zone but the maximum inhibition zone was shown by *C. verum* with a value of 96.00% and the minimum inhibition by *Cocos nucifera* with a value of 66.48% occurred.

**Keywords:** Incidence, Fire blight of apple, Balochistan, Management, essential oils.

Corresponding Author: Muhammad Waris

Email: [waris.faqir@gmail.com](mailto:waris.faqir@gmail.com)

© 2025 Faculty of Agriculture, UPR. All rights reserved.

### Article history

Received: January 24<sup>th</sup>, 2025

Revised: April 26<sup>th</sup> 2025

Accepted: April 30<sup>th</sup> 2025

### INTRODUCTION

Apple (*Malus domestica*) is the main fruit crop of Balochistan. This fruit belongs to the *Rosaceae* family. Almost 6000 apple varieties have been described in the literature, and only a few of them are important. The apple tree is a temperate, deciduous tree that grows well in temperate climates where most commercial varieties meet the required cooling temperature. Some of the tropical and subtropical countries are in demand to produce their own leafy fruits. Although this fruit tree originates from temperate regions of the world, their environmental needs

can be regularly met by this region (Syed et al., 2006). The apple is most popular fruit in the world after mango, which contains antioxidants, vitamins, fiber and much other nutrition. Because of this nutritious value of apple, it can help prevent a number of health problems.

Apple has different varieties different color and shapes which provide a variety of beneficial minerals (Potassium 145mg, Phosphorus 12mg, Magnesium 6mg, Calcium 7mg and Sodium 3mg) content per 100g. Nutrients that can help to human i.e. they can reduce different difficult conditions of human health like reduce heart disease, obesity, diabetes,

cancer and many several other diseases (Jillian et al., 2019). The apple is one of the most famous fruits of Southwest Asia and European countries. It is found in temperate parts of the world and coldest large mountains of subtropical areas. It was first revealed in the Caucasus that it spreads in Europe, rapidly in early times. From Europe, apples spread out through South America United States and Australia. Various shapes of apples cultivated in Pakistan from a long time immemorial, especially in the places near Afghanistan, China and Iran. The apple is a temperate fruit and is really grown in coldest regions, needs a cool temperature for its proper growing and progressive growth. Blossom stage required maximum chilling period in winter season for best fruits setting. (Malik et al., 2018).

In Pakistan various forms of apple showing great diversity in shape, size, color and taste. Cultivation of apples after mango in Pakistan is limited only to the mountainous regions of Balochistan, high altitudes of Khyber Pakhtoonkhwa and Punjab. Balochistan produces a wide variety of quality fruits and vegetables. From a horticultural point of view, the fruits are in quite reasonable graduated volumes. Balochistan is prominent for its fruits and hence it is called the fruit basket of Pakistan. This province excelled in producing a number of dry and fresh fruits, some of which may not be grown anywhere in Pakistan. Worldwide, it grows relating 5 million hectares and produces to 83 million tons of average yield every hectare of 17 tons. Pakistan cultivated on 95.3 thousand hectares and produced 670 thousand tons of apples with an average yield of 7.0 tons. Balochistan cultivated 88,807 hectares producing 527,642 tons with an average yield per hectare of 5.9 tons. In apple production, Balochistan is one of the major contributors and its gives 85.05% share in production in the country. This is according to GOP (2019-2020). The main benefit of planting an apple orchard in areas under lower altitude of warm regions is that the fruits may reach market earlier and therefor results getting better price. Besides, the beneficent number of orchards are susceptible to large insects and pathogen attacks, as insects and fungi multiplies in warm climate very fastly (Muhammad and Malik, 2018). Apples have many diseases like Fire blight (*Erwinia amylovora*), scab disease (*Venturia inaequalis*), Ripe rot (*Rhizopus arrhizus*), sooty spot (*Gloeodes pomigen*), Downy mildew (*Podocphaera leucotricha*), and Root rot (*Phytophthora cactorum*), and white root rot (*Rosellinia necatrix*). In fact, the most devastating bacterial disease of apple trees is a fire blight caused by *Erwinia amylovora*. It infected the trees of *Rosaceous* family, some species belong to genera *Malus*, *cydonia*, *pyrus*, *sarbus*, *Amelanchier*,

*Crataegus*, *cotoneaster sorbus* *Eriobotrya*, *Photinia*, *Mespilus*, *Raphiolepis*, *Heteromeles*, *Pyracantha* and *Stranysaesia* some other are reported as susceptible host of *E. amylovora* "unusual circumstances", like genera *Rosa*, *Aruncus*, *Spirea*, *Prunus* and *Fragaria* (Pegg et al., 2013). The casual organism of fire blight disease of apple *E. amylovora* is a gram-negative enterobacterium, the inoculum of the bacterium survive in diseased tissue of the leaf margins of cankers. They become active when climate are suitable in spring season with rainfall, during this period pathogen reached to a threshold level when temperature is 18.3°C (Dewdney et al., 2007).

Disease losses 68 million dollars were caused by fire blight of apple in northwest America and New Zealand about 10 million dollars in one area. In the year (2000), in Italy and Lebanon about 500,000 trees were demolished. *Erwinia amylovora* is a devastating disease of apple trees. Destroying flowers and shoots, cobwebs and often trees, the losses caused by the pathogen are estimated at more than 42 million dollars. The losses are so severe and affect the apple trees badly (Islam et al., 2014). In apple orchards, fire blight caused great losses. Financial damages included reduced apple yields, tree death, and the cost of removing infested trees and timber and replanting orchards. Due to fire blight every bud wood per farm was lost 20 to 40% productive approximately. In the season of summer, the diseased trees removal big labor after applied repeated application when symptom was shown (Srdan et al., 2017). While keeping in view the effects of fire blight of apple. The proposed study was conducted at Balochistan to observed the incidence of fire blight of apple its ecofriendly management.

## MATERIAL AND METHODS

### Survey and sample collection

Surveys of the orchards to examine number of trees were affected by the pathogen *E. amylovora* and how many of them were resistant and healthy so the research was performed during summer season 2020. Many different orchards of Apple were surveyed in different districts of Balochistan Killa Abdullah, Pishin, Quetta and Mastung on the basis of symptoms of fire blight of apple in the area of each district; consist of different number of trees. Among those districts Killa Abdullah was examined with having four orchards consisting of 180 trees. Quetta was examined with having four orchards consisting of 80 trees. Pishin was examined with having four orchards consisting of 100 trees. Mastung was examined with having four orchards consisting of 80 trees. From these tested trees symptomatic samples were taken for testing as a sample randomly. The

sample was cut with the help of sample cutter sassier and wrapped in newspaper and labels each sample. Collected samples were brought to Plant Pathology Department of Balochistan Agriculture College Quetta. Calculated of disease incidence, isolation, purification, identification and management of the pathogen. Incidence of fire blight was determined by the following formula prescribed by Khamis & Sergio (2020).

Disease incidence (%)

$$= \frac{\text{Apple trees showing disease parts}}{\text{Total number of tested apple trees}} \times 100$$

### Isolation, purification, and preservation

Bacterium *E. amylovora* isolation was performed on agar nutrient media and second one is leaven medium, containing yeast extract 1-g, NaCL 2.5-g, peptone 2.5-g, sucrose 25-g then dissolved in 500-ml distilled water, this solution was prepared for autoclave. pH 7.0 to 7.2 was adjusted, place it 121°C, 15psi for 15 minutes in autoclaved for sterilization. After sterilization the heated media transfer to laminar flow

for pouring in petri plates then wait for solidification of media in plates, the small pieces samples were transfer to plates and place in incubator for 2 to 3 days at 27°C, for bacterial colony growth when the growth was appear on plats then transfer single colony with help of sterilized loop from suspected growth colony on the new agar nutrient plates the it has been prepared with peptone 20g and then incubate for also 2 to 3 days at 27°C, for the growth of bacteria.

*E. amylovora* suspected growth colonies were studied on basis of cell morphology, characterization like size, shape, elevation, color, edge on the surface. Repeated this operation five more time for insurance. Prepared pure culture for further identification. Colony morphology was kept under observation to identify plant pathogenic bacterium on nutrient agar medium. Furthermore, bacterial gram staining test was performed at 39 °C and 4% NaCl containing NA medium. In addition, a fluorescence assay was applied the plates were incubated at 366nm after 48 hours under UV light (Islam et al., 2014).

Table 1: Survey areas.

| Localities/Districts | Number of Orchards / Nurseries | Total No of Trees | No of Tested Trees/Samples |
|----------------------|--------------------------------|-------------------|----------------------------|
| Killa Abdullah       | 4                              | 180               | 5                          |
| Quetta               | 4                              | 80                | 5                          |
| Pishin               | 4                              | 100               | 5                          |
| Mastung              | 4                              | 80                | 5                          |

### Biochemical characterization

The procedure for biochemical characterization of the isolates was carried out essentially as outlined by Maheshwari et al. (2006). This test was performed.

#### Gram staining

Gram staining was different staining manner which was used to distinguish bacterium gram-negative or gram-positive species of physical and chemical interaction on their cell walls, for preparation of smear placed one after those 1 or 2 drops of culture on clean slide and heat. 1 or 2 drops to fix of crystal violet solution was placed on the fixed smear and then washed with sterile distilled water. Gram iodine B solution was applied for 1 minute and then washed with 95% alcohol. Finally, the smear was counterstained with safranin for 30 seconds and washed again with sterile water. The air-dried coating was then examined under a light microscope after applying oil immersion. A purple appearance indicated the presence of gram-positive bacteria, while a pink to red appearance indicated the presence of gram-negative bacterial growth.

The observation slide was left in the water bath for 5 minutes. Then thoroughly washed and counterstained by adding safranin for 30 seconds. It was washed again and then dried and observed under an oil immersion microscope objective (Maheshwari et al., 2006).

#### Catalase Test

The test assay catalase was performed hydrogen peroxide drop coated bacteria were placed on microscopic slide. Mixture was formed foam or bubbles that bacterium was designated as positive, and mixture doesn't show bubbles or foam, that bacteria designated as "catalase-negative" (Vincent et al., 1970).

#### Amylase Test

Amylase test determined whether the bacteria is able to break down starch into maltose through the activity of the extracellular enzyme amylase. A sterile swab or sterile loop was used to pick a few colonies from pure culture dish. A starch sheet was drawn in a line across the width of the sheet. Several cultures tested on one agar plate, each with a marked line, or the plate divided into four quadrants for this

purpose. Plates were incubated at 37°C for 48 hours. 2-3 drops of 10% iodine solution were added directly to the edge of the colonies. Than wait for 10-15 minutes and results were recorded positive test. Negative test; The medium w stained dark to the edge of the isolated colonies, two species were inoculated onto a starch plate and incubated at 30°C until growth was seen (left panels). The petri plate was then filled with iodine solution and photographed after 10 minutes (right panel). Amylase-positive species showed a clear halo around growth (upper

growth line). Amylase-negative species did not have that clear halo of lower growth lines (Basirie, 2006).

#### Preparation and of Essential Oils with different Concentration

Efficacy of different essential oils under in-vitro condition against *E. amylovora* were tested the concentration of the oils were made as Five prepared essential oils Cinnamon oils, Clove oils, Sesame oils, Coconut oils and Rose oils obtained from laboratory of Balochistan Agriculture College Quetta.

Table 2: Essential oils used against *Erwinia amylovora* and their concentration.

| Treatment | Essential Oil | Botanical Name             | Concentration |     |     |
|-----------|---------------|----------------------------|---------------|-----|-----|
| T1        | Cinnamon oil  | <i>Cinnamomum verum</i>    | 5%            | 10% | 15% |
| T2        | Sesame oil    | <i>Sesamum indicum</i>     | 5%            | 10% | 15% |
| T3        | Rose oil      | <i>Rosa indica</i>         | 5%            | 10% | 15% |
| T4        | Clove oil     | <i>Syzygium aromaticum</i> | 5%            | 10% | 15% |
| T5        | Coconut oil   | <i>Cocos nucifera</i>      | 5%            | 10% | 15% |
| T6        | Control       | -                          | 5%            | 10% | 15% |

#### Evaluation of Antibacterial Activities of essential oils under invitro condition

The method was used for determination of antibacterial activities of different essential oils with it fractions that is a paper disc diffusion method. In this method sterilize filter paper disc 5mm Whatman No1 was impregnated in essential oils 20 µL, fractions related. Carvacrol and thymol dissolved as well as standard solutions then placed on the center of plate which having nutrient agar 20 containing 20 ml of inoculated nutrient agar 100 µl consisting 10 CFU ml-1 bacterium suspension. Clear mobile phase was used as a negative control. Dishes were incubated at 27°C for 48 hours. Using a compass for the measurement of inhibition zone around paper disc. Test was performed three times (Basim et al., 2006). Different concentration of each essential oils were prepared according to their Quantity on the basis of 5%, 10% & 15%, first concentration was 5% from each essential oil; for Preparation of 5% solution mixed 5 percent essential oils to 95 percent distilled water the mixture was prepared for nutrient agar inoculating plate. Second concentration was 10% from each essential oil, for solution preparation 10 percent essential oils was mixed to 90 percent distilled water, the mixture was prepared for applying on nutrient agar inoculating plate. The third concentration was 15% from each essential oil; for solution preparation 15 percent essential oils was mixed to 85 percent distilled water the mixture was prepared for nutrient agar inoculating plates for control of plant pathogenic bacteria *E. amylovora* cause disease fire blight of apple

(Birol et al., 2010).

#### Statistical analysis

Disease incidence was analyzed from the number of affected trees and the percentage within the survey field of trees. The collected data were statistically analyzed using the computer software "Student edition of Statistix, version 8.1.

### RESULTS AND DISCUSSION

#### Disease Incidence

Disease was observed in all four surveyed locations of Balochistan, data in Figure 1 shows that the disease incidence (%) in four (4) districts of Balochistan Killa Abdullah, Pishin, Quetta and Mastung varies. In District Mastung the disease incidence was found high i.e. 18.75% as compared to other locations followed by killa Abdullah 16.43%, Quetta 13.33% and Pishin 12.50%. The pathogen of fire blight of Apple was highly transmissible where rainfall and dew factors was high. These environmental factors provide favorable conditions were seen for the pathogen which cause infection. Researchers indicated in their research that different environmental factors like high temperature, rainfall and dew favored of bacteria to cause disease (Khamis & Sergio, 2020).

#### Isolation, Identification and morphological characterization of *E. amylovora*

*Erwinia amylovora* was observed for morphological characteristics of colonies and cells. *E. amylovora* is the Gram-negative rod shape bacteria, pathogenicity was found

between normal and filamentous cell population. The morphologically studies of the bacterium cell growth colony was performed by incubate the colony at 28°C when it appeared on NA media. The appearance of the colonies showed a circular, domed shape, white smooth colonies were found (Jones & Geider 2001). The identification of bacteria was done based on the morphological characterization of colony growth, shape, color of the colony. The morphological studies of *E. amylovora* colonies

were done after the incubation of colony when it appeared on NA medium at 28 C. The appearance of the colonies showed a circular, domed shape, white smooth colonies were found (Islam et al., 2014). Microscopic study was done to identified the causal agent of fire blight of apple on the basis of cell morphology characterization of the 20 bacterial isolates. Cell morphology was observed. Rod shape motile gram negative bacteria was observed which indicated that the bacteria is *E. amylovora*.

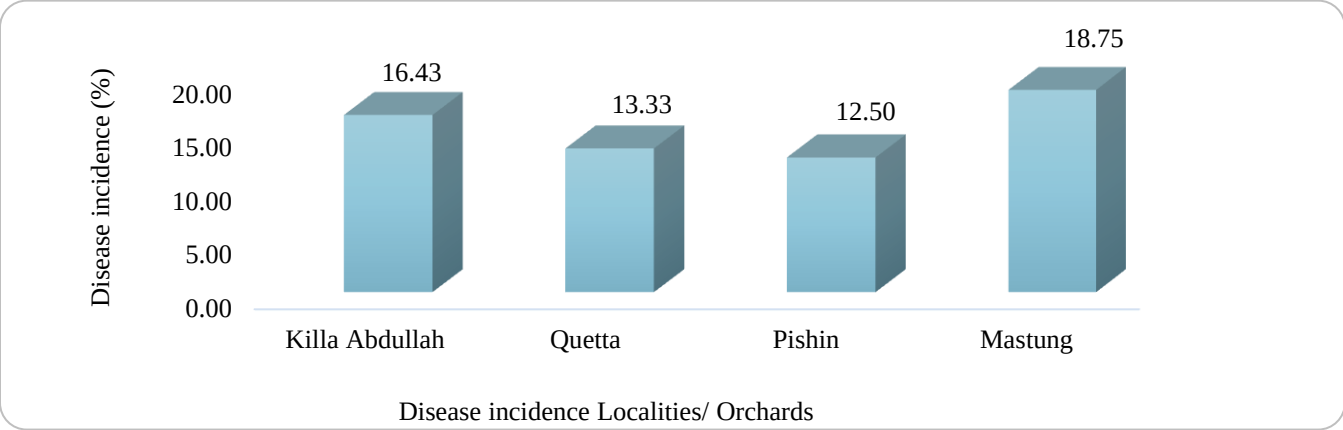


Figure 1: Disease incidence of fire blight pathogen (*E. amylovora*).

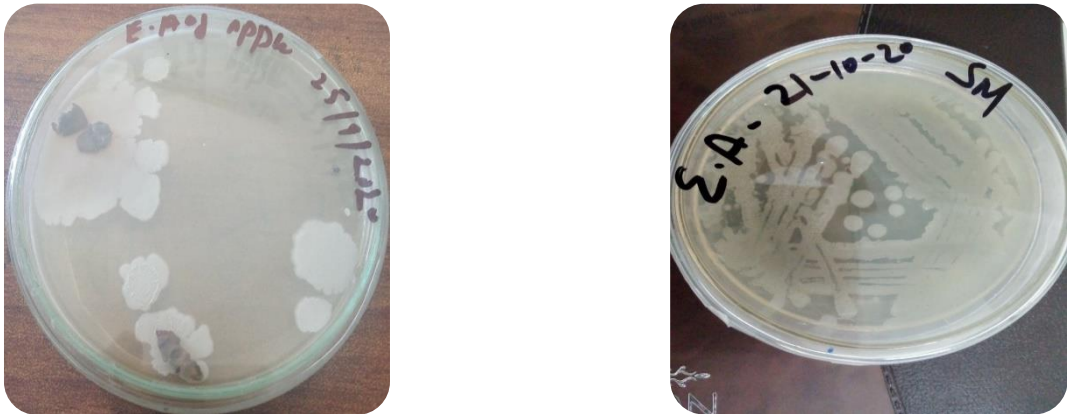


Figure 2: A) Isolation of pathogen B) cultural characteristics.

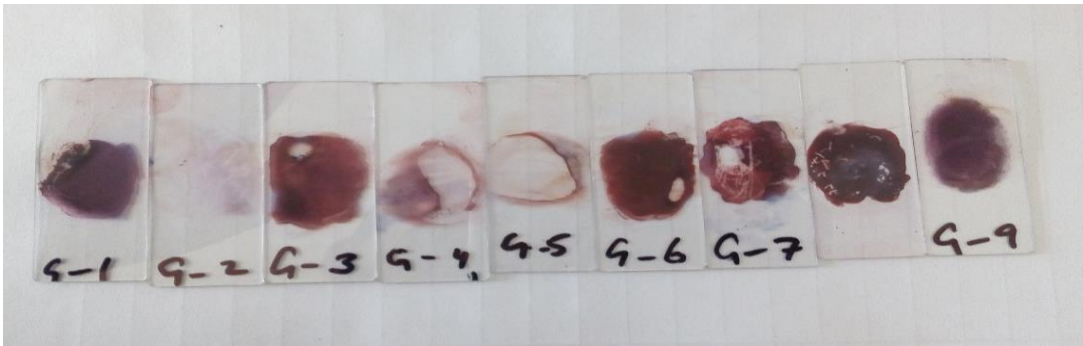


Figure 3: Slide preparation during gram staining test to distinguish bacteria.

The main purpose of the research was to quick and accurate identification and discrimination of *Erwinia amylovora* apple blight. The test was performed by observing reaction of hydrogen peroxide drops on microscopic slide with appearance of bacterium seamer and checked the status of the test whether it is catalysis positive or negative appearance. If bubble or foam

emerging then catalase-positive. After applying hydrogen peroxide it was proven that the catalase test is positive. In Amylase test addition of iodine solution directly 2-3 drops of 10% to colony edges then wait for 10 to 15 minutes result was recorded that the medium was change color to dark around on edge the colony (Basirie & Rossi 2006).

Table 3: Catalase and amylase test.

| Strain No | Catalase Test (+/-) | Amylase Test (+/-) |
|-----------|---------------------|--------------------|
| S No.1    | +                   | -                  |
| S No.2    | +                   | -                  |
| S No.3    | +                   | -                  |
| S No.4    | +                   | -                  |
| S No.5    | +                   | -                  |
| S No.6    | +                   | -                  |
| S No.7    | +                   | -                  |
| S No.8    | +                   | -                  |
| S No.9    | +                   | -                  |
| S No.10   | +                   | -                  |
| S No.11   | +                   | -                  |
| S No.12   | +                   | -                  |
| S No.13   | +                   | -                  |
| S No.14   | +                   | -                  |
| S No.15   | +                   | -                  |
| S No.16   | +                   | -                  |
| S No.17   | +                   | -                  |
| S No.18   | +                   | -                  |
| S No.19   | +                   | -                  |
| S No.20   | +                   | -                  |

**Inhibition zone for control of *Erwinia amylovora* at 5% concentration using different essential oils**

In Table 4 the result show that all Essential oils Cinemon oil, Sesame oil, Rose oil, Clove oil, Coconut oil and also showed inhibition zone means that they inhibit the bacterial growth among all the essential oil the Cinemon oil at 5% concentration showed the maximum control over bacterium *Erwinia amylovora* with value 16.08 millimeter while coconut oil at 5% concentration showed minimum inhibition zone with value 11.96 millimeter over pathogen. In control no inhibition

zone was observed. The Cinemon oil at 5% concentration had the maximum control over pathogen *Erwinia amylovora* in comparison with the coconut oil, which showed minimum inhibition zone with value of 11.96 millimeter over the pathogen. Similar results were also reported by other authors who tested the effect of essential oils against other bacteria (Lacobellis et al., 2005). Phenolic compound of essential oils have ability dissolved with bacterial membrane, and enter inside the cell interact with cellular metabolic mechanism then disturb it. (Blanka et al., 2007).

Table 4: Comparison test for growth of inhibition zone at 5% concentration using different essential oils.

| Essential Oil | Botanical Name          | Diameter of Zones of Inhibition (Hrs) |       |       |       | Mean of Inhibition Zones (mm) |
|---------------|-------------------------|---------------------------------------|-------|-------|-------|-------------------------------|
|               |                         | 24                                    | 48    | 72    | 96    |                               |
| Cinemon       | <i>Cinnamomum verum</i> | 15.5                                  | 16.5  | 16.8  | 16.25 | 16.08a                        |
| Coconut       | <i>Cocos nucifera</i>   | 11.5                                  | 11.75 | 12.08 | 13.00 | 11.96d                        |
| Clove         | <i>Syzygium</i>         | 11.5                                  | 12.00 | 12.17 | 13.00 | 12.17cd                       |

|         |                        |       |       |       |       |        |
|---------|------------------------|-------|-------|-------|-------|--------|
|         | <i>aromaticum</i>      |       |       |       |       |        |
| Rose    | <i>Rosa indica</i>     | 12.25 | 12.92 | 13.5  | 13.75 | 13.10c |
| Sesame  | <i>Sesamum indicum</i> | 13.00 | 14.25 | 14.25 | 15.5  | 14.25b |
| Control |                        | 0     | 0     | 0     | 0     | 0.00e  |
| LSD     |                        |       |       |       |       | 0.97   |

#### Inhibition zone for control of *Erwinia amylovora* at 10% concentration using different essential oils

The result show that all Essential oils i.e. Cinemon oil, Sesame oil, Rose oil, Clove oil, Coconut oil at 10% concentration showed significant result. Amongst all the essential oils the Cinemon oil at 10% concentration showed the maximum control over pathogen *Erwinia amylovora* with value 17.75 millimeter and coconut oil at 10% concentration showed minimum control inhibition zone in diameter with 12.99 millimeter over pathogen while no inhibition zone was seen in control treatment. The result show that the Cinemon oil at 10% concentration showed the maximum control over pathogen *Erwinia amylovora* while coconut oil at 10% concentration showed minimum control inhibition zone with 12.99 millimeter over pathogen in a Table 5 In harmony with the result of researcher experiment, in which the essential oils O.valare, O.

compactum and T. Vulgaris were most effective against *E. amylovora* as compare to dictamnus and Majoram which were considered to be effective (Daffera et al., 2003). In our study Essential oils of 10% concentration Cinemon and Sesamum more effective other than the 5% concentration. Some authors have shown in research that the plant phenolic volatile substances carvacrol and eugenol reduce virulence pectobacteria and inhibit their quorum sensing. Characteristics of E. oils used as effective pesticide safely uses, healthy practice, low risk to human health, cheaper cost, paved a way for plant life and hazard to environment instead of antibiotics. (Karmi et al., 2010 & Zarubova et al., 2015)). Generally Cinnamon essential oils showing more effectiveness for different bacterial strains, clove oils less effective other than cinnamon oils used different concentration for the control of Pectobacterium carotovorum (Matej et al., 2017).

Table 5: Comparison test for growth of inhibition zone at 10% concentration using different essential oils.

| Essential Oil | Botanical Name             | Diameter of Zones of Inhibition (Hrs) |       |       |       | Mean of Zones of Inhibition (mm) |
|---------------|----------------------------|---------------------------------------|-------|-------|-------|----------------------------------|
|               |                            | 24                                    | 48    | 72    | 96    |                                  |
| Cinnamon      | <i>Cinnamomum verum</i>    | 17.00                                 | 17.75 | 18.00 | 18.25 | 17.75a                           |
| Coconut       | <i>Cocos nucifera</i>      | 12.63                                 | 12.75 | 13.25 | 13.33 | 12.99c                           |
| Clove         | <i>Syzygium aromaticum</i> | 13.5                                  | 13.75 | 13.92 | 14.5  | 13.92b                           |
| Rose          | <i>Rosa indica</i>         | 13.75                                 | 13.75 | 14.17 | 15.00 | 14.17b                           |
| Sesame        | <i>Sesamum indicum</i>     | 13.25                                 | 13.92 | 14.25 | 14.25 | 13.92b                           |
| Control       | -                          | 0                                     | 0     | 0     | 0     | 0.00d                            |
| CV            |                            |                                       |       |       |       | 0.54                             |

#### Inhibition zone for control of *Erwinia amylovora* at 15% concentration using different essential oils

In Table 6 the result at 15% concentration revealed that all treatment used i.e. Cinemon oil, Sesame oil, Rose oil, Clove oil, Coconut oil have significant effect on pathogen *Erwinia amylovora* but the maximum effect was carried out by Cinemon oil with value 20.16 millimeter while coconut oil at 15% concentration showed minimum control of inhibition zone with value of 14.67 millimeter over pathogen in compare to control treatment where no any effect seen. In this research that higher effective of *Cinnamomum verum* oil was found

with maximum inhibition zone against colony growth *E. amylovora* in vitro condition at 15% concentration with value of 20.16 millimeter followed by Sesamum indicum with value of 15.83 millimeter on high concentration the both indicated maximum inhibition zone. In our study, cinnamon oils have antibacterial effect, which could be caused by euganol in combination with benzyle benzovate both from cinnamon leaf essential oils (Ali et al., 2002 & Joshi et al., 2016). Some authors have shown in research that the plant phenolic volatile substances Euganol and carvacrol prevents quorum sensing in pectobacteria and essential oil can be safely used

as effectious pesticides with minimum risk to human being health and the environment (Zarubova et al., 2015).

Table 6: Comparison test for growth of inhibition zone at 15% concentration using different essential oils.

| Essential Oil | Botanical Name             | Diameter of Zones of Inhibition (Hrs) |       |       |       | Mean of Zones of Inhibition (mm) |
|---------------|----------------------------|---------------------------------------|-------|-------|-------|----------------------------------|
|               |                            | 24                                    | 48    | 72    | 96    |                                  |
| Cinemon oil   | <i>Cinnamomun verum</i>    | 19.5                                  | 20.00 | 20.17 | 21.00 | 20.16a                           |
| Coconut oil   | <i>Cocos nucifera</i>      | 14.00                                 | 14.67 | 14.75 | 15.25 | 14.67c                           |
| Clove oil     | <i>Syzygium aromaticum</i> | 14.75                                 | 15.00 | 15.1  | 15.8  | 14.98c                           |
| Rose oil      | <i>Rosa indica</i>         | 15.5                                  | 15.5  | 15.50 | 16.25 | 15.69b                           |
| Sesame oil    | <i>Sesamum indicum</i>     | 15.33                                 | 16.00 | 16.00 | 16.00 | 15.83b                           |
| Control       |                            | 0                                     | 0     | 0     | 0     | 0.00d                            |
| CV            |                            |                                       |       |       |       | 0.47                             |

**Inhibition percentage (%) with different concentrations of essential oils against *Erwinia amylovora***

In overall inhibition (%) of essential oils reaction between various essential oils and their effect on bacterial colony growth on agar plates in vitro condition, Totally six treatment were used in order to show their efficacy in which one of them was kept as control where essential oils was not applied. All the essential oils showed antibacterial activities against the pathogen *Erwinia amylovora* on all three concentration 5%, 10%, 15% but High efficacy showed on 15% concentration as compared to lower concentrations. Essential oil Cinemon was seem more effective against the pathogen followed by Sesame, Rose, Clove and Coconut. Cinemon oil was excellent in reduction of bacterial colony growth when applied at the concentration 5%, 10%, 15% respectively. Furthermore, in inhibition zone percentage the

efficacy of all essential oils and their effect on bacterial colony growth on agar plates *In-vitro* condition were tested. All the essential oils showed better antibacterial activities against the pathogen *Erwinia amylovora* on all three concentration 5%, 10%, 15%. But Cinemon showed high effect on pathogen 96.00% while Coconut oil show least effect 66.48% but there is no inhibition zone was found in control treatment. Moreover, Arif et al., (2025) expressed that The essential oils showed better antibacterial activities against the bacterial pathogen *Dickeya dadantii* in all three concentrations 5%, 10%, 15%. Furthermore another work expressed that there *Syzygium aromaticum* showed a good antibacterial activity and the inhibited the growth of *E. amylovora* with inhibition zone diameter ranging from 21 to 27 millimeter at 20% *in-vitro* condition, (Kubilay, 2020).

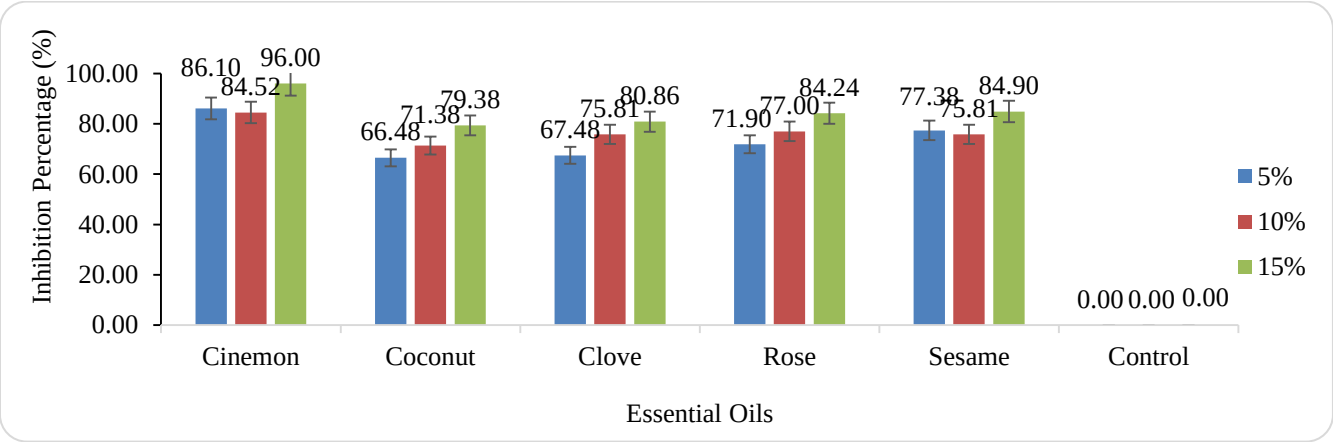


Figure 4: Inhibition percentage (%) with different concentrations of essential oils.

**CONCLUSION**

It was observed that the maximum incidence of fire blight was recorded at Mastung. Followed by Quetta, Pishin and

Killa Abdullah. Invitro evaluation of Essential oils showed that *Cinnamomun verum* at 15% concentration maximum inhibition zone against colony growth of bacterial pathogen,

followed by *Sesamum indicum*, *Rosa indica*, *Syzygium aromaticum*, and *Cocos nucifera* having 96.00%, 84.90%, 84.24%, 80.86% and 79.38% zones of inhibition respectively. It can be concluded that this study proved Antimicrobial test of essential oils against *E. amylovora* are efficient to develop the inhibition zone and minimize the pathogen. Moreover, *C. verum* at 15% concentration inhibit the colony growth of bacterial pathogen and recommended against fire blight disease caused by *E. amylovora*. Further research is required on large scale.

### COMPETING OF INTEREST

The authors declare no competing interests.

### AUTHORS CONTRIBUTIONS

The authors declare that there is no conflict of interests regarding this publication.

### REFERENCES

- Ali, N.A., Mohtar, M., Shaari, K., Rahmani, M. & Ali, A.M. (2002) 'Chemical composition and antimicrobial activities of the essential oils of *Cinnamomum verum* Gamb', *Journal of Essential Oil Research*, 14(2), pp. 135–138.
- Arif, M., Waris, M., Sadiq, S.A., Lutf, A., Jabeen, Z., Ahmed, I., Shah, J., Khan, K. & Samad, A. (2025) 'Evaluation of essential oils for in-vitro management of *Dickeya dadantii* causing soft rot of onion', *Phytopathogenomics and Disease Control*, 4, pp. 87–92.
- Basim, E., Basim, H. & Ozcan, M. (2006) 'Antibacterial activities of Turkish pollen and propolis extract against plant bacterial pathogens', *Journal of Food Engineering*, 77(4), pp. 992–996.
- Basirie, A. & Rossi, M.P. (2006) 'Different bacterial biochemical tests for identification of bacterial strains', *Journal of Plant Disease and Protection*, 36(7), pp. 1123–1127.
- Birol, O., Fatih, S., Mustafa, O. & Mehmet, M.O. (2010) 'The antibacterial activity of essential oil of oregano (*Origanum vulgare* L.)', *Journal of Food, Agriculture and Environment*, 8(2), pp. 272–274.
- Blanka, K. & Roman, P. (2007) 'Effectiveness of plant essential oils on the growth of *Erwinia amylovora*, the causal agent of fire blight disease', *Pest Technology*, 19(7), pp. 23–144.
- Daffera, J.D., Ziogas, N.B. & Polission, G.M. (2003) 'The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis*', 23(12), pp. 415–419.
- Dewdney, M.M., Biggs, A.R. & Turechek, W.W. (2007) 'A statistical comparison of the blossom blight forecasts of MARYBLT and Cougarblight with receiver operating characteristic curve analysis', *Phytopathology*, 97(22), pp. 1164–1176.
- Government of Pakistan (2019–20) *Agricultural Statistics of Pakistan*. Ministry of Food, Agriculture and Livestock, Economics Wing, Islamabad, Pakistan.
- Islam, M.A., Alam, M.D., Urmee, S.A., Rahman, M.H. & Razu, M.H. (2014) 'Isolation, identification, in vitro antibiotic resistance and plant extract sensitivity of fire blight causing *Erwinia amylovora*', *Journal of Plant Pathology and Microbiology*, 5(1), pp. 233–235.
- Jillian, K.M.S. (2019) 'Medically reviewed on apple benefits, nutrients, antioxidants, and medical', *Journal of Applied Science*, 6(2), pp. 432–434.
- Jones, A.L. & Geider, K. (2001) '*Erwinia amylovora* group', in: *Laboratory guide for identification of plant pathogenic bacteria*. 3rd ed. St. Paul, MN: APS Press, pp. 40–54.
- Joshi, J.R., Khazanov, N., Senderowitz, H., Burdman, S., Lipsky, A. & Yedidia, I. (2016) 'Plant phenolic volatiles inhibit quorum sensing in pectobacteria and reduce their virulence by potential binding to ExpI and ExpR proteins', *Scientific Reports*, 8(6), pp. 38–40.
- Karami, O.R., Khodaverdi, M. & Ali-Akbari, F. (2010) 'Antibacterial effect of effective compounds of essential oils against *Erwinia amylovora*', *Journal of Agricultural Sciences and Technology*, 12(6), pp. 35–45.
- Khamis, Y. & Sergio, R.R. (2020) 'Premature apple fruit drop: associated fungal species and attempted management solutions', *Horticulturae*, 27(6), pp. 31–33.
- Kubilay, K.B. (2020) 'Management of *Erwinia amylovora* by potential bio-pesticides in vitro and in vivo condition', *Department of Plant Protection, Faculty of Agriculture, Selcuk University*, 8(1), pp. 38–45.
- Lacobellis, N.S., Pietro, L.C., Francesco, C. & Felice, S. (2005) 'Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils', *Journal of Agricultural and Food Chemistry*, 53(7), pp. 57–61.
- Maheshwari, D.K., Dubey, R.C. & Kang, S.C. (2006) *Biotechnological application of microorganisms: a*

- techno-commercial approach*. New Delhi: I.K. International Publishing House.
- Malik, M.A. & Muhammad, A.S. (2018) 'Horticulturist', *Fruit Production*, 33(10), pp. 3–4.
- Matej, B., Pavel, P. & Klovcek (2017) 'Chemical composition and antimicrobial activity of cinnamon, thyme, oregano and clove essential oils against plant pathogenic bacteria', *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 65(4), pp. 1129–1134.
- Muhammad, A.S. & Malik, M.A. (2018) 'Assistant horticulturist', *Fruit Production*, 42(1), pp. 124–127.
- Pegg, K., Coates, L., Cooke, T., Forsberg, L. & Manners, A. (2013) *Fire blight: a biosecurity threat to the Australian nursery industry*. Available at: [www.ngia.com.au](http://www.ngia.com.au).
- Srdan, G.A. & Christopher, L.M. (2017) 'Reduction of *Erwinia amylovora* with dormant copper sprays in blossom blight control', *Cornell University New York State Agricultural Experiment Station, Highland and Geneva, NY*, 25(10), pp. 344–348.
- Syed, M.K., Muhammad, N.S. & Syed, A.S.S. (2006) 'Production constraints of apple in Balochistan', *Journal of Applied Sciences*, 34(6), pp. 167–173.
- Vincent, J.M. (1970) *A manual for practical study of root nodule bacteria*. IBP Handbook No. 15. Oxford: Blackwell Scientific Publishers.
- Zarubova, L., Kourimska, L., Zouhar, M., Novy, P., Douda, O. & Skuhrovec, J. (2015) 'Botanical pesticides and their human health safety: the example of *Citrus sinensis* essential oil and *Oulema melanopus* under laboratory conditions', *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 65, pp. 89–93.