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Bio-control of citrus canker by soil microorganisms

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A B S T R A C T

Citrus canker is a serious disease caused by bacterial pathogen *Xanthomonas Aaxonopodisprv.* Citri occurs in large areas of the world citrus growing countries including India. Nearly 40% of India's total land is used for citrus agriculture. Citrus fruits are known for their excellent nutritional value and ability to both present and cure a number of ailments. A pathogen that causes citrus canker was identified in a sample of ill citrus fruit. Citrus canker causing pathogen was isolated from diseased citrus fruit sample. Identification of pathogen causing citrus canker was done by morphological and biochemical characterization. Isolation of bacteria against citrus canker by using serial dilution method.

The isolation of pathogen of citrus canker was done successfully. Bacterial pathogen causing citrus canker was isolated on nutrient agar media and showed yellow colourd colony. In the microscopic characterization of bacterial pathogen showed Gram negative, rod shape. In case of morphological characterization, yellow pigmentation, entire margin, circular shape and convex elevation was observed. On the basis of morphological, biochemical and microscopic characterization bacterial pathogen causing citrus canker identified as *Xanthomonas Aaxonopodisprv. citri.*

The isolation of soil microorganisms for control of *Xanthomonas Axonopodispv. citri.* causing citrus canker was done on Nutrient Agar media. Different rhizosphric soil samples of banana, basil, Hibiscus, Ashoka and citrus were collected and isolation of bacteria was done. Bacterial isolates from banana and citrus hizospheric soil samples were showed positive control against Xanthomonas axonopodispv. citri.

Keywords : *Citrus, Citrus Canker, Pathogen, Control*

1. Introduction:

The genus citrus is one of the most important fruit crops worldwide. It belongs to family Rutaceae comprising 140 genera and 1300 species distributed throughout the world it is a long lived perennial crop and is grown in more than 100 countries across the world. India is the world's sixth-largest producer of citrus fruits. Spain, the United States, Israel, Morocco, South Africa, Japan, Brazil, Turkey, and Cuba are other nations that produce citrus. In terms of fruit output in

India, it comes in third place, behind banana and mango. In India, citrus makes up around 40% of the entire area planted to citrus crops. The mandarin (*citrus reticulata*) citrus species is the most significant commercial variety in India. Citrus fruits have well documented nutritional and health benefits they can help prevent and cure some disease. Citrus were historically used for their high content of Vitamin C. Citrus fruit juices such as oranges lime and lemon may be useful for lowering the risk of specific type of kidney stones. Citrus canker (*Xanthomonas citri sub sp. citri. Xec*) is currently thought to be endemic in Florida. People, equipment, and plant material are just a few of the surfaces that can get polluted. Decontamination is done to lessen the possibility of bacterial spread by people or equipment in both disease-endemic and disease-free settings [1].

Importance of citrus fruits apart from daily consumption of citrus fruits as such or squash or cordial or as jam or marmalade from the juice and pulp. Citro flavinoids are extracted from the peel of citrus fruits as a byproduct in factories, where citrus juices are extracted to prepare squashes and cordials. They also contain essential oils and vitamin C. The major symptom of citrus canker infection is the corky lesions that develop on the leaves, stems, shoots and fruit between 7-10 days after infection. In severe cases the disease also leads to shoot dieback, defoliation premature fruit drop general tree decline citrus canker is not systemic it causes local lesions only. The diseased plants are characterised by the occurrence of conspicuous raised necrotic lesions that develop on leaves, twigs and fruits [2].

The appearance of canker lesions can vary depending on the citrus variety, plant part affected and the age of the lesions. Lesions can be irregular in shape and appear atypical if found in association with a wound site or citrus leaf miner (Phyllonistiserella) feeding Lesions can also appear atypical if trees are water stressed through drought or reduced irrigation. In this case the lesions can appear flatter than normal due to reduced turgor in the plant tissues. The disease first appears as tiny dark, slightly raised spots usually on the lower leaf surface. As they age, they change colour from tan through brown to grey and protrude from both leaf surfaces. The lesions expand and become thick and spongy or corky, developing a water-soaked margin and often surrounded by a chlorotic halo. The water-soaked margin may disappear as lesions age and it is not as prominent on resistant host cultivars. Lesions are usually visible on both sides of the leaves and eventually the centres of the lesions become crater-like or may fall out, creating a shot hole effect. Infection can lead to early leaf fall. After infection of the leaves, symptoms typically spread onto twigs and eventually to branches. As the lesions age and thicken, they become corky and may develop a brown crater-like depression in the centre. Cankers can occur on woody stems, bark may develop discoloured areas, and dieback of stems coupled with internal reddening can occur. Infected fruit may develop scabs or pitting. Typical lesions initially resemble large oil glands on the rind. These gradually darken and become cork-like in texture. They are usually round, and can occur either singly or in groups. Fruit may drop prematurely. Lesions on fruit that have been through the packing shed appear less corky and erumpent than lesions found on un-waxed fruit. During processing the top of the lesion is shaved off leaving a smooth, slightly raised dark spot, still with an irregular margin [3].

A collection of about 27 different bacterial species known as Xanthomonas are responsible for 400 different plant diseases. Since Xanthomonas bacteria thrive best at temperatures about 30°C, warm, humid climates are particularly problematic for them. Through the breathing pores (stomata) and water-releasing pores (hydathodes) on the leaf surface, bacteria infiltrate plant leaves. Plant damage caused by insects and machinery opens up wound entry locations for the bacterium Xanthomonas. Xanthomonas bacteria typically have a yellow hue. From the Greek words Xanthos, which means “yellow,” and monas, which means “entity,” comes the phrase Xanthomonas. Commercial items are manufactured

using *Xanthomonas compestris.* It generates xanthan gum, which is found in several toothpastes and low-fat yogurts and is thicker: Gram-negative rod-shaped proses bacteria with a single polar flagella are called *Xanthomona Citri* [4].

Biological control pests with their natural enemies, including parasites, predators, diseases and competing organisms is called biological control, management of certain plant beneficial microorganism's biological control agents The (BCAS) seems to be a promising and environmental friendly method to control plant pathogens. Controlling plant diseases is necessary to preserve the quantity and quality of food, feed, and fiber that producers worldwide produce. To avoid, lessen, or manage plant diseases, several strategies may be employed. Aside from using effective horticultural and agronomic techniques, growers frequently largely depend on chemical pesticides and fertilizers. The remarkable increases in agricultural productivity and quality over the past 100 years have been largely attributed to such inputs. However, fear-mongering by certain opponents of pesticides and environmental degradation from excessive and improper use of agrochemicals have prompted significant shifts in public perceptions toward pesticide usage in agriculture. The use of chemical pesticides is now strictly regulated, and there is political pressure to take the most dangerous compounds off the market. Moreover, due to the potential scale at which chemical applications may need to be made, the proliferation of plant diseases in natural ecosystems may make such applications unsuccessful. In order to combat pests and diseases, several researchers studying pest management have concentrated on creating substitutes for synthetic chemicals. In order to combat citrus canker, various soil bacteria were isolated for use as bio-control agents in the current investigation [5].

2. Literature review:

It has been studied that management of citrus canker on acid time *Bacillus Subtilis* in West Bengal India the pattern of citrus Canker disease development and its progress was observed through year round disease monitoring studies [1].

Khodakaramian and researchers in 2008 investigated that the particular Pseudomonas bacterial strain's antagonistic effectiveness against *Xanthomonar axonopodispv* [2]. *Citri,* the causative agent of citrus bacterial canker disease, was studied in greenhouse and laboratory settings. The outcomes of in vitro tests demonstrated that every bacterial strain exhibited a range of antagonistic activities that were suppressive on Xanthomonascitri. In the greenhouse studies involving the application of antagonistic bacterial strains to suppress xanthomonasaxonopodipov, the most efficacious strains were chosen and assessed. Citrus bacterial canker disease is caused by Citri, a potentially promising strain that decreased the amount of illness spots from 23.8 to 64.0% [2].

Faruk Hasan and researchers in 2017 isolated and characterized *Xanthomonas axonopodispv. citri* bacteria in Citrus aurantifolia canker disease and evaluation of its antibiotic susceptibility, antibacterial sensitivity and antagonistic activity [3]. Isolated bacterium was characterized by different biochemical test method. Isolated bacterium was found to be gram negative, small size, rod shaped, motile and pink in colour. The biochemical tests showed catalyse positive, glucose fermenting, lactose non-fermenting and urease test negative. The antibiotic susceptibility, antimicrobial sensitivity and antagonistic activity were determined by disc diffusion method. Gentamycin showed highest 21.0±0.0 mm diameter of zone of inhibition against the isolated bacteria. Allium sativum extract showed highest 19.9±0.4 mm diameter of zone of inhibition against the isolated bacteria. The highest antagonistic activity was found 17.410.3 mm diameter of zone of inhibition by soil [3].

Pal and reserarchers in 2006 studied that to preserve the quantity and quality of food, feed, and fiber supplied by growers worldwide, plant diseases must be managed [4]. Several strategies may be applied to avoid, lessen or manage plant diseases, Growers frequently rely significantly on chemical pesticides and fertilizers in addition to sound

horticultural and agronomic procedures. Over the past 100 years, there have been notable advancements in crop output and quality, mostly attributed to the use of such agricultural inputs. However, people's perceptions of the use of pesticides in agriculture have changed significantly as a result of the environmental contamination brought on by the overuse and misuse of agrochemicals, as well as the scare tactics used by certain opponents of the practice. Political pressure is present to remove the most dangerous chemicals from the market, and there are stringent limits on the usage of chemical pesticides nowadays. Furthermore, due to the potential scale at which chemical applications may need to be made, the proliferation of plant diseases in natural environments may make such applications unsuccessful. As a result, several researchers studying pest management have concentrated on creating substitutes for artificial pesticides in the management of pests and illnesses. The so-called biological controls are one set of these substitutes [4].

Shah and reserarchers in 2014 reported 11 species of Citrus found and used in India have been identified and discussed with their photographs [5]. In brief the citrus fruits used during Akbar the Great time are also presented with their market values of that period [5].

Gade and Lad in 2018 studied that citrus is the third largest fruit sector in India, behind banana and mango, and plays a significant role in the country's horticulture wealth and economy. At 8.7 million tons, the nation currently ranks sixth in the world for citrus output, behind China, Brazil, the United States, Spain, and Mexico. Numerous bacterial, viral, and fungal diseases pose a hazard to citrus crops; however, in central India, *Xanthomonas axonopodispv.* citri-caused bacterial citrus canker and several Phytophthora spp.-caused fungal diseases are the most destructive. In citrus from central India, Phytophthora spp., which include P. parasitica, P. citrophthora, and P. palmivora, are the cause of gummosis, collar rot, and root rot. Among these, at least three different varieties or kinds of citrus canker are known. Canker A, often known as the Asiatic form, is the most devastating and affects most major citrus cultivars in our area. A few possible biological agents were looked into to help control these serious illnesses. *Bacillus subtilis and Pseudomonas* fluorescens, two species of Trichoderma, have been discovered to be useful in the treatment of various illnesses[6].

Robert and reserarchers in 1991 studied that in Florida, citrus is affected by two diseases that are both brought on by xanthomonails, a type of bacteria. One is comparable to the illness known as Asiatic citrus canker (also known as canker A), which was declared extinct in the state in 1931. After the illness had been absent from the state for more than 50 years, samples of infected oranges were gathered and discovered in 1985. Canker. A was most likely in the state for a few years prior to 1986, however it's hard to say for sure how long. In its brief history, the other illness has also been referred to as canker and nursery canker. Presently, it is known as bacterial spot of citrus. After being discovered for the first time in 1984, it was classified as a distinct variety of citrus canker[8].

Das and reserarchers in 2003 studied all the agricultural pests and diseases that threaten citrus crops, citrus canker is one of the most devastating. The disease, caused by the bacterium *Xanthomonas axnopodis pe citri*, occurs in large areas of the world's citrus growing countries including India. At least 3 distinct forms or types of citrus canker are recognized. Among these, Asiatic form (Canker A) is the most destructive and affects most of the major citrus cultivars. A severe case of the disease can cause defoliation, dichack, highly blemished fruit, poor fruit quality, and early fruit drop, among other impacts. The illness is aided by a warm, muggy, gloomy climate, a lot of rain, and high winds. In nations or areas where the disease is not prevalent, measures to control canker include tight and ongoing field surveys, the prompt removal of diseased trees, and quarantines or regulatory programs that forbid the introduction of infected citrus plant material and fruit. When canker is present in a nation, the most efficient ways to combat the disease are usually integrated systems of compatible cultural practices and phytosanitary measures that include resistant hosts, eliminating

inoculum sources, carefully planning windbreak systems, and applying protective copper-containing and/or antibiotic sprays on time.The identification of pathogens and strains linked to the disease, their interactions with hosts, and the molecular mechanisms underlying their pathogenicity are all reviewed in this work. Aspects of epidemiology and management techniques [9].

Mondal and reserarchers in 2014 studied that a severe illness affecting acid lime [*Citrus aurantifolia* (*Christm.) Swingle*], citrus canker is caused by *Xanthomonas axonopodispvcitri* (Hasse) Vauterin. It affects people all over the world, including West Bengal, India. The disease is mostly dependent on its secondary dissemination by raindrop splashing, mechanical contact during storms, and small citrus leaf injury (*Phyllocnistiscitrella Stainton*). The standard recommendation for managing citrus canker involves the application of agrochemicals in the form of sprays along with antibiotics. On the biological control of the illness, not much research has been published. In 2009–2010, an inhibitory strain of Bacillus subtilis (5–12) was used in an experiment conducted in a farmer's field (an acid lime orchard) in Nadia, West Bengal. Five hatches (6 numbers of plants/ batch) of plants were sprayed with a single aqueous suspension (2.7 x 109 cells/ml) of bacterial cells, leaving four hatches unsprayed. Using a 0–4 scale, the percent disease index (PDI) of both treated and untreated plants was measured at each month throughout the year. Prior to a week of spraying, the first PDI was also obtained. A single application of the bacterial suspension in July, when the sickness is at its worst, has sufficiently reduced the illness. Twenty days following treatment, there was a noticeable reduction in the illness, suggesting that the spore-forming bacteria may have seized control of the plant surfaces [10].

3. Methodology:

3.1 Diseased sample collection:

Figure 1 shows the diseased sample of citrus canker

Fig. 1. Diseased citrus fruits were collected from local market of Aurangabad

3.2 Isolation of pathogen causing citrus canker disease.

Collected diseased lemon samples and cut the diseased part of lemon with the help of scalpel. Surface sterilized it with 0.1% mercuric chloride (add 0.1 gram mercury chloride in 100ml distilled water).Then washed it with sterile distilled water for three times. Kept samples for Oozing out bacteria for 10 minutes. Nutrient agar media was prepared, the media was poured in petri-plates and streaking was done. Plates are incubated at 28 to 30°C for 2 to 3 days.

3.3 Identifications of Pathogen Causing Citrus canker Disease:

Identification of causal organism of citrus canker was done on the bases of morphological, Biochemical and microscopic characterization Biochemical Identification of bacterial isolates was done by using Bergey's Manual. Morphological characterization: The pathogen causing citrus canker were primarily identified based on morphological characteristics like colour, size, margin, elevation and microscopic observation.

3.3.1 Gram's staining

Thin smear of bacterial culture was made on clean glass slide. Air dried and heat fixed. Smear wax covered with crystal violet for 60 seconds Slide was washed with distilled water Smear was covered with Gram's iodine solution for 60 seconds Slide was

washed with 95% ethyl alcohol and then distilled water Again the smear was covered with safranin for 30seconds Washed with distilled water and blot dried Air dried. Observed under microscope.

3.4 Biochemical analysis various tests are given.

3.4.1 Catalase test:

Nutrient agar was prepared.The medium was poured into culture tubes and flasks.

It was sterilized by autoclaving at 15lb pressure for 15 minutes. The nutrient agar slants were inoculated with test organism. An inoculated nutrient agar slant was kept as control. The cultures were incubated at 28°C for 24 hrs. After incubation the slants were flooded with 1ml of 3% hydrogen peroxide (H_2O_2) and observed for the formation of gas bubbles. The occurrence of gas bubbles scored positive for catalyse production.

3.4.2 KOH Test :

Thin smear of bacterial culture was made on clean glass slide. The drops of KOH solution was added on the bacterial smear.String like appearance was observed.

3.4.3 Mucoid growth on NA +5% glucose.

Nutrient agar media with 5% glucose were prepared and autoclaved at 15 lb pressure for 15 minutes. Nutrient agar media with 5% glucose was poured in petri plates and allow to solidify. Culture of bacterial pathogen was straked on nutrient agar medium The plates were kept for incubation at 28°C for 24 to 48 hours

3.4.4 Gelatin Hydrolysis:

Gelatin media was prepared and sterilized using autoclave at 121°C. Stab inoculation was made using inoculating loop, From each culture into its appropriately labelled deep tube of Nutrient gelatin. The tubes were incubated at 28°C for 4 to 7days After incubation, the tubes were placed in refrigerator at 4°C for 15 minutes. The tubes were observed for liquification.

3.4.5 Fluorescence test:

King's B medium was prepared .Pour that media in Petri plates. bacterial pathogen was streaked on Petri plates Incubate it in incubator at 30 for 2days. Observed the Fluorescence of bacteria under UV illuminator

3.5 Bio-control of bacterial pathogen causing citrus canker by soil Bacteria :

Different Rhizosperic soil sample were collected from different plants for control of bacterial pathogen causing citrus canker e.g, Banana, Sarcoaasoca, citrus, Basil were selected for isolation of bio-control bacteria.

3.5.1 Isolation od Soil Microorganism:

Test tubes were taken and distilled water was added in test tube The serial dilutions of soil sample were made from 10 to 10 Serial dilutions were spread on nutrient agar plate.The Plates were kept for incubation at 28°C Next day the colonies were selected and inoculated in test tube containing nutrient broth. Test tubes were kept for incubation at 28°C for 24-48 hours. The disc of filter paper were dip in that Nutrient Broth. Placed the disc on plate of Xanthomonas. The plates were kept for incubation for 2 to 3 days at 28 to 30°C. Next day the plates were observed.

3.5.2. Dise diffusion method for bio-control of bacterial pathogen causing citrus canker by soil bacteria-

Sterilized nutrient media were poured in petri plates. Petri-plates were kept for solidification After solidification citrus canker causing bacteria *Xanthomonas auxonopodispro citri* were streaked on plain nutrient agar plate. Already prepared broth culture of isolated soil bacteria used as bio-control of xanthomonas were taken Disc of filter paper were dipped in prepared broth culture of test soil bacterial isolates and disc were kept on streaked plate of *Xanthomonas axonopodispv citri.* Plates were kept for incubation for 24-48 hour at 28°C.

4.Result :

4.1 Isolation of bacterial pathogen caused by citrus canker disease.

Diseased citrus sample caused citrus canker were collected from local Aurangabad market. Bacterial pathogen caused citrus canker disease isolated by using nutrient agar media. For isolation 28°C temperature is required and incubation period was 24 to 48 hours. Colonies of bacterial pathogen further purified on nutrient agar and kept for preservation on nutrient agar slants.The isolation of pathogen of citrus canker was done successfully (Fig. 2)

4.2 Identification of causal organism of citrus canker

Identification of causal organism of citrus canker was done on the basis of morphological, biochemical and microscopic characterization.

4.2.1 Morphological characterization:

In case of morphological characterization, bacterial pathogen causing citrus canker was observed for pigmentation, colony shape, elevation, margin and size of colony. Bacterial pathogen was showed yellow pigmentation of nutrient agar media, circular filliform colony shape, convex elevation, entire margin and medium size of colony (Table 1 and Figs. 2,3).

Table 1- Morphological characteristic of bacterial pathogen causing citrus canker.

Pigmentation	Colony shape	Elevation	Margin	Cell shape
Yellow	Circular-filliform	Convex	Entire	Single Rod

4.2.2 Microscopic Characterization

Bacterial pathogen causing Citrus canker were observed under Microscopicon 10x, 40x, 100%. Gram negative and Rod shaped Bacteria were observed under microscope Rod (Cocobacilli) shaped bacteria in single rod were observed.

4.2.3. Biochemical Characterization

Bacterial pathogen causing citrus canker were observed for biochemical tests viz Catalase, gelatine hydrolysis, mucoid formation, yellow pigmentation, non-fluorescence, string formation. In case of catalase test, bacterial pathogen showed positive result by showing gas bubble formation (Figs.4,5 & Table 2). In case of gelatine hydrolysis, bacterial pathogen showed positive result by showing gelatine liquification when kept at 4°C. (Figs.5 & Table 2) In case of KOH test, bacterial pathogen showed positive result by showing string formation. (Figs.5 & Table 2) In case of fluorescence test, bacterial pathogen showed negative test by showing non fluorescence under UV-Transilluminator. (Figs.7 & Table 2) In case of growth on nutrient agar plus 5% glucose, bacterial pathogen shows yellow pigmentation with mucoid colony formation after 48hrs at 28+2°C. (Figs.6 & Table 2) On the basis of morphological, biochemical and microscopic characterization, bacterial pathogen causing citrus canker identified *Xanthomonas axonopodis pe citri.*

Table 2 Biochemical characterization of bacterial pathogen causing citrus canker.

Sr. No.	Test name	Result of Xonthomonas aronopodisPycitri
1	Catalyze Test	Positive
2	Gelatine hydrolysis test	Positive
3	Fluorescence test	Negative, non-fluorescence
4	Nutrient agar+ 5% glucose	Mucoid colony with yellow pigmentation
5	KOH test	Positive
6	Nutrient agar test	Yellow colonies

Bio-control of *Xanthomonaspvcitri* by bacterial isolates and rhizospheric soil samples was observed.

In case of bacteria soil bacteria, isolate (B1) banana was showed 10 mm diameter for control of *Xanthomonas axonopodispv citri*

Table 3 Control of Xanthomonas axonopodispvcitri

Sr. no.	Soil bacterial isolate	Biocontrol by soil bacterial isolate (mm)
1	Isolate CI	10 mm
2	Isolate BI	10 mm

4.3 Bio-control of Bacterial pathogen causing citrus Xanthomonas axonopodispvcitri by soil bacteria.

Different plate rhizospheric soil sample were collected from ashoka banana, citrus, Tulsi.By using serial dilution method different soil bacterial isolates were taken to test against *Xanthomonas axonopodispv citri.* with help of disc diffusion method, rhizospheric soil bacterial isolates of ashoka, banana, citrus, Tulsi plant were positive control against *Xanthomonas axonopodispv citri.*

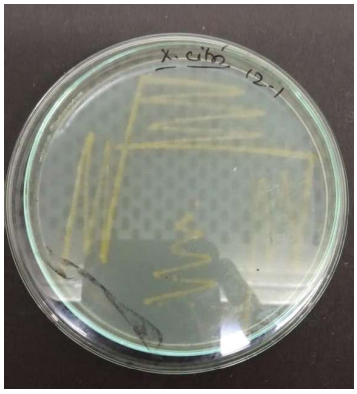

Fig. 2 - Bacterial pathogen of citrus causing citrus canker

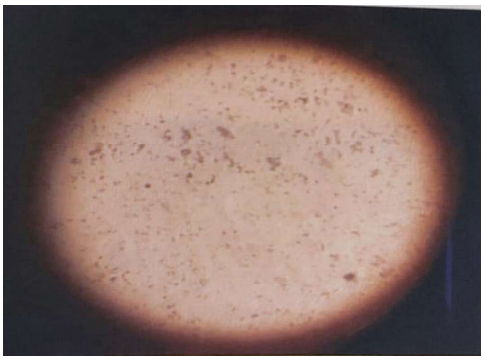

Fig. 3 - Microscopic observation of bacterial pathogen causing citrus canker

Fig. 4 - Catalyze test

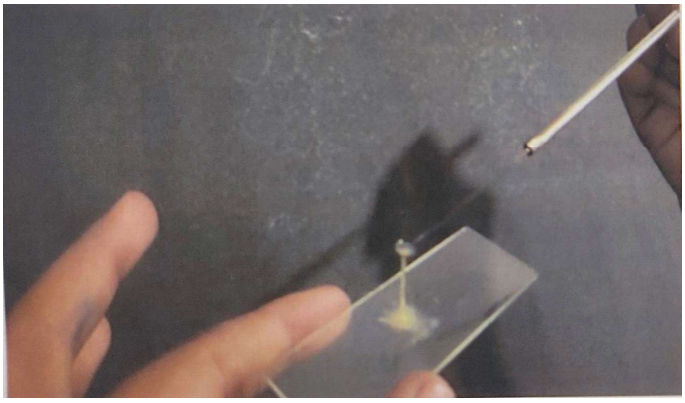

Fig. 5 - KOH Test

Fig. 6 - Yellow pigmentation with mucoid growth

Fig. 7 - Gelatin hydrolysis Test

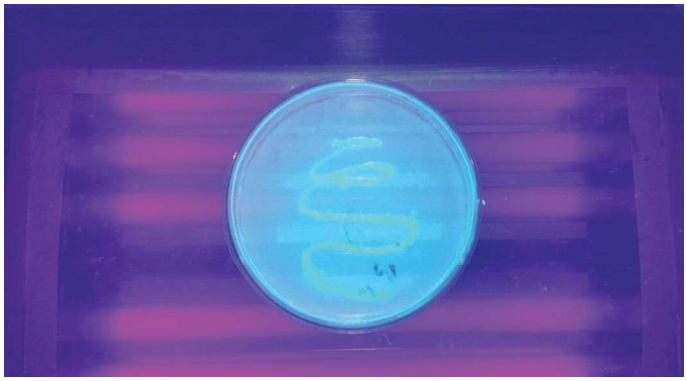

Fig. 8 - Fluorescence Test

Fig. 9 - Bio-control of
Xanthomonas axonopodispvcitri by soil bacteria

5. Discussion

The genus citrus is one of the most important groups of fruit crops world-wide belong to family Rutaceae. The citrus canker is a serious disease of citrus fruit crop. Biological control is needed to control citrus canker. Controlling pest with their natural enemies including parasites, predators, diseases competing organism is called biological control.

In the presence study, isolation of bacterial pathogen causing citrus canker is done by using diseased lemon sample and identification of pathogen is done based on microscopic characterization using gram staining and biochemical characterization like catalase test positive. Similarly, Hasan researchers in 2017 studied that isolated bacterium was found to be gram negative, small size, rod shaped, motile and pink in colour. Hasan has also found positive result of catalase test.

In the present study isolation of soil bacteria is done by using serial dilution and antagonistic activity of soil bacteria is studied by using measuring diameter of control by soil bacterial isolate. Similarly, Hasan 2017 have studied that the highest antagonistic activity was found to be 17.4±0.3 mm diameter of control by soil bacteria.

In the presence study certain soil bacteria suppressed the growth of bacterial pathogen causing citrus canker. Similarly, Das in 2014 studied that antagonistic strain of soil bacteria *Bacillus subtilis* control the activity of bacterial pathogen causing citrus canker.

The genus citrus is one of the most important groups of fruit crops world-wide, belongs to family Rutaceae, controlling pests with their natural enemies, including parasites, predators, diseases and competing organisms is called biological control.

Importance citrus fruits are apart from daily consumption of citrus fruits as such or squash or cordial or as jam or major symptom of citrus canker infection the corky lesions that develop onthe leaves, stems, shoots and fruit between 7-10 days after infection. In severe cases the disease marmalade from the juice and pulp Citro flavonoids are extracted from the peel of citrus fruits as a by-product in factories, where Citrus juices are extracted to prepare squashes and cordials. They also contain essential oils and vitamin C.

It is investigated that antagonistic activity of selected pseudomonas bacterial strain against bacterial canker disease was carried out in laboratory and greenhouse conditions. [4].

Diseased citrus fruits sample collected from local market of Aurangabad isolation of pathogen causing citrus canker disease is done by using diseased lemon sample identification of pathogen causing citrus canker disease is done. By using microscopic observation and biochemical characterization. Isolation of bio- control of bacterial pathogen causing citrus canker by soil bacteria.

6. Conclusions :

The bacterial pathogen causing citrus canker were isolated on nutrient agar Media. For identification of bacterial pathogen causing citrus canker in morphological and microscopic characterization yellow pigmentation, circular margin, covex elevation, rod shape was observed. In biochemical characterization catalase test positive, gelatin hydrolysis test positive, fluorescence test negative mucoid colony formation positive and KOH Test positive were observed. Biochemical characterization isolated bacterial pathogen causing citrus canker were identified as *Xanthomonas axonopodispvcitri* Ashoka, banana citrus, tulsirhizospheric soil bacterial isolate were foundpositive to control of *Xanthomonas axonopodispvcitri*

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