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# Control and cause of bacterial blight disease/telya on pomegranate

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**Abstract**
One of the major threat on Pomegranate cultivation in India, measurely in Southern Part and Western Part is Bacterial disease caused by *Xanthomonas axonopodis pv punicae*. The currently used chemicals or other control measures for this disease are not showing fruitful effects against this organism. Hence cost effective and microbial originated method along with botanical agent as well as chemical agents for controlling the bacterial growth and spread. Also the time required for inhibiting the growth of organism due to action of different agents showed less than 1 hr required for chemical and botanical origin. Also have shown microbial originated substance ie *Lactobacillus* cell free crude extract requires less than 3 hr that is higher time required but might less hampered on the quality of fruit and nutrition. The chemicals usage hamper nutritional and quality of the fruit.

**Keywords:** Pomegranate, bacterial blight disease, control measures

## 1. Introduction
Pomegranate (*Punica grantum*) is an important fruit crop with export potential. It is a species of fruit bearing devious shrub belonging to family *Punicae* (*Lathraceae*). (Hosamano *et al.*, 2016) [3] Pomegranate is medicinally and economically important fruit crop. The pomegranate tree is native from Iran traveled to the Himalayas in Northern India and has been cultivated since ancient times throughout the Mediterranean region of Asia, Africa, Europe. Earlier pomegranate tree was free from most of the Diseases and pests but due to changes in the enviroment leading to cause changes in the disease occurrence. This is one of the favourite table fruit of tropical and subtropical region. Hence increase in disease occur naive with respect to time has increased the need for study the disease causative agent and it's control.
The disease observed to be caused on pomegranate leads to cause severe losses which leads to give an economic downturn in case of Pomegranate sales. As the increased demand for the pomegranate fruit due to it's medicinal as well as nutritional aspects. This leads to increase the lands under pomegranate cultivars. As increased production indicates increase in requirement for the protection of cultivars from disease causative agent and increasing it's nutrition.
According to Horticulture Board of India, the total area under cultivation of Pomegranate in India is 131.00 thousand ha and product is around 1346.00 thousand tons in 2013-14. Maharashtra is the leading producer of pomegranate contributing to about 70.2% of pomegranate production followed by Karnataka, Gujarat, a Andhra Pradesh and Tamilnadu.
Pomegranate crop is prone to number of diseases among which bacterial blight is serious problem and threat due to high epidemic potential. The disease was first reported in India from Delhi in 1952 and later from Bangalore in 1959. The disease was of minor importance until 1991, when it appeared in epidemic proportion at IIHR experimental plot at Bangalore, leading to 60-80% crop losses. Further the outbreak was noticed in pomegranate growing areas of Karnataka and western Maharashtra mainly in Solapur district and disease observed for throughout the year.
Also the disease caused during harvesting thus leading to severe losses in production. Hence required to look for the solution. Also the control measure for the same is nor yet observed as the disease can spread from infected harvest to the healthy ones via plant to plant contact, runoff water, rains or rain water splashes, contaminated tools and insects. After every shower the incidence of disease causing increases.
As the antibacterial agents or pesticides or other fertilisers used are unable to control the disease severity as well as the disease spreading.

## 2. Materials and methods

### 2.1. Collection of sample

The part of the pomegranate plant to be studied is Fruit. The variety of the pomegranate fruit is Bhagwa which has been selected from Western Maharashtra Village, Dighanchi, District Sangli and are collected during end of December 2019 that is Autumn crop. The number of samples collected during this season was three and another round of collection of sample being done during mid of February 20th which is end of Autumn crop. As in case of Western Maharashtra, the disease prevalence in Sangli district observed to be on Mrudula. But for my study we have selected very common and consumer demanding Bhagwa variety. Total number of sample being studied are 5.

### 2.2. Isolation and characterisation of organism

#### 2.2.1. Sample preparation

The surface sterilisation of the samples colected is done by using 10% $HgCl_2$. The piece of the fruit is cut from sample and put in a sterilised petri plate and tease the inner core part of the sample.

#### 2.2.2. Isolation from the sample

From the teased area and inoculate it on Nutrient Agar plate medium. (Himedia Labs Pvt Ltd) and incubated at Room Temperature (R.T.) for 48-72 hrs. Screen for Yellow coloured mucoid medium to large colonies. Also perform Gram staining method for confirmation of Gram negative rods only being selected for further study. Also perform catalase test with $H_2O_2$. (Sigma Labs) so as observing effervescence on contact for positive result by culture

#### 2.2.3. Species confirmation

The isolated organism have been further confirmed by using biochemical testing VITEK systme Version 08.01. (Suburban Diagnostics)

### 2.3. Antibiotic susceptibility test

The AST has been performed by Kirbey Bauer method that is Agar cup method by using Nutrient Agar (Himedia Labs Pvt Ltd) and antibacterial agents selected are of different origins, inclusive of botanical, chemical and Microbial.

### 2.4. Preparation of extracts or antibacterial agents

#### 2.4.1. Chemical compounds

C-1 Alcohol: Regularly used for laboratory purposes.
C-2 Sulpex: Sulphur containing agent which is used as regular fertiliser. As decrease in fertility leading to decrease in yield and hence in disease caused. This fertilizer is being used to maintain sulphur contain and that too maintain the growing minerals required.
C-3 Streptocycline (5000ppm) and
C-4 Streptocycline (10000ppm)

#### 2.4.2. Botanical compounds

B-1 Cold water neem extract: Dried neem leaves mixed in water at regular temperature. (traditional methods)
B-2 Hot neem extract water based: Neem leaves are being boiled in water to prepare an extract. (Traditional methods)
B-3 Cold Methanol extract *Hibiscus brackenridgei* (Yellow Hibiscus) flower: The flower of the selected plant species has been selected to study antibacterial property. The flower has been allowed to dry (Sun drying) grinded and this extract has been prepared by addition of Methanol cold extraction by shaker conditions. Kept on shaker for 72 hrs till the half volume reduces. And the liquid extract used for testing. As the extraction from other plant part may lead to interferance of other material.
B-4 Cold Acetone extract *Hibiscus brackenridgei* (Yellow Hibiscus) flower: In the prepared Methanol extract Acetone is added and allowed to evaporate to half of the volume added. (ref)
B-5 Dr. Batra'a hand sanitizer: Tulsi based hand sanitizer. (Commercial botanical agent)

#### 2.4.3. Microbial extract

M-1 Soil collected from Sathaye ground, isolated *Bacillus* extract in Nutrient broth with 5% Glucose. And incubated for 48 hrs and centrifuged. Supernatant collected and studied for antibacterial activity of *Bacillus* producing secondary metabolites and its efficacy against pathogen.
M-2 Pyocyanin: *Pseudomonas aeruginosa* collected from Sathaye College showing pigmentation (green). Seed on Cetrimide Agar (HiMedia). Incubate at 37°Cfor 96 hrs.
Wash surface with st. Distilled and cut agar in 1 cm pieces and dissolve in 20 ml dry Chlorofrom. Shake vigorously and remove Chloroform by pipette. Filter this and dissolve in 0.2 M HCl and gives red colour due to Pyocyanin and yellow colour - discard. Add 0.2 M NaOH drop by drop to the red coloured solution till Blue colour appear. Add 20 ml Chloroform vigorously shake. And use as a pyocyanin antibacterial substance.
M-3 *Lactobcillus* extract: *Lactobacillus* spp. isolated from Idli batter on MRS Agar (Hi Media). Incubate isolate in MRS broth and incubate under microaerophillic condition for 48 hrs. This broth was centrifuged and supernant collected. This collected supernant was used for the production of bacteriocin i.e. antibacterial substance.
M-4 *Bacillus* (contaminant) obtained from the plate and inoculate in st. Nutrient broth and incubated at R. T. for 48 hrs and centrifuged so as to collect supernant and hence used to stufy for antibacterial property of the extract.
M-5 *Bacillus* extract isolated from garden soil and inoculate in st. Nutrient broth and incubated at R. T. for 48 hrs and centrifuged so as to collect supernant and hence used to stufy for antibacterial property of the extract.

### 2.4 Contact time inhibition assay

The selected chemical, microbial and botanical agents tested for antibacterial properties then tested for the time required for inhibitory effect on the isolate. The agents have selected out of them having best activity. Hence selected tested against isolate. The spot from the solution being touched on the Nuterint agar plate. Incubate at R. T. for 48 hrs.

### 2.5 *In vivo* study

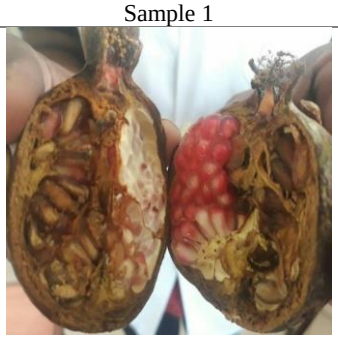
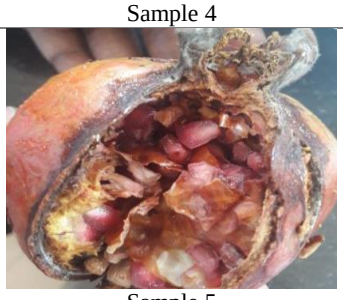
By surface sterilising pomegranate the puncture in the has been made and then swab of each isolate has been done on alternate side on a single pomegranate. Then this has wait for few hrs after which the antimicrobial agents have been sprayed and incubated at R. T. for certain weeks and record the changes.

## 3. Results

### 3.1. Sample selected analysis

As the samples collected are observed to be showing cracking, and lesions also shows the disease progression due to foul smell. As the blackening at the site of cracks observed leads to confirm the H2S production and hence indicates Sulphur reducing organisms. The lesions observed on the fruit and also leading to blackening around the lesion.

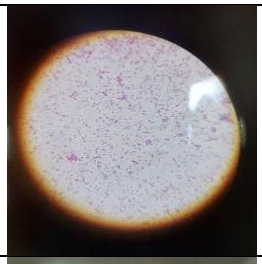
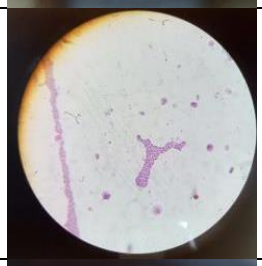
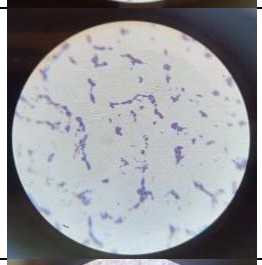
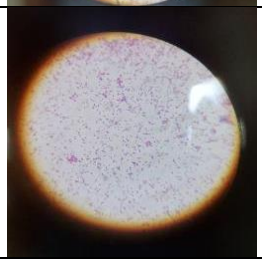
**Table 1:** Sample observation and symptoms

| Sample | Observation |
|---|---|
|  Sample 1 | Blackening on the suface and lesion observed. Also blackening of the inner seedlings. Foul smell. |
|  Sample 2 | Blackening of inner core completely and degraged. Only one lesion observed. |
|  Sample 3 | Lesion observed on the surface and also blackening of inner part. |
|  Sample 4 | Blackening and lesion observed on the surface and leading to cause infection and also drying of the inner core observed |
|  Sample 5 | Crackening of the fruit and also fungal growth is being initiated. Also slight blackening of inner core observed. |

### 3.2. Isolation and characterisation of isolates

The set of organism have been obtained following mentioned characters which shows the resemblance to the causative agent.

**Table 2:** Cultural characteristics of Isolated pathogen

| Sample no. | Isolate no | Characteristics | | | | |
|---|---|---|---|---|---|---|
| | | Colour | Consistency | Gram nature | Catalase | |
| Sample 2 | I | Yellow | Mucoid | Gram negative rods | Positive |  |
| Sample 2 | VII | Yellow | Mucoid | Gram negative rods | Positive |  |
| Sample 1 | IX | Yellow | Mucoid | Gram negative rods | Positive |  |
| Sample 1 | XIII | Yellow | Mucoid | Gram negative rods | Positive |  |
| Sample 5 | XV | Yellow | Mucoid | Gram negative rods | Positive |  |
| Sample 5 | XVI | Yellow | Mucoid | Gram negative rods | Positive |  |
| Sample 4 | XXI | Yellow | Mucoid | Gram negative rods | Positive |  |

### 3.3 Identification of Isolates by using Biochemical tests: (Bergey's manual)

**Table 3:** Results for biochemical tests

| Sugars fermentation | I | VII | IX | XIII | XV | XVI | XXI |
|---|---|---|---|---|---|---|---|
| Arabinose | + | + | + | + | + | + | + |
| Galactose | + | + | + | + | + | + | + |
| Cellobiose | + | + | + | + | + | + | - |
| Trihalose | + | + | + | + | + | + | + |
| Mannose | + | + | + | + | + | + | + |

### 3.4 Identification of Biochemical tests by using VITEK System

Out of 18 isolates 6 isolates have been selected on results obtained from biochemical tests. The results have shown that the different types of organisms being observed which are natural flora of soil and one might be a coliform.

Xap I: *Streptomonas maltophilia*
Xap VI: *Streptomonas maltophilia*
Xap IX: *Streptomonas maltophilia*
Xap XIV: *Spingomonas paucimobilis*
Xap XV: *Streptomonas maltophilia*
Xap XXI: *Pantoe spp.*

Hence this indicates that these might be the organisms present in the sample along with the *Xanthomonas axonopodis pv Punicae*

### 3.5 Antibacterial susceptibility test by Kirbey Bauer method

The antibacterial activity of selected agents against the isolated organism have been studied and shown various activity and thus confirms the selected compound has antibacterial properties or not. (Agent names material and method)

**Table 4:** Antibacterial susceptibility test of the isolates against Selected Botanical extracts by Kirbey Bauer method carried in duplicates

| Botanical agent | Isolate no and zone of inhibition (mm) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV | XVI | XVII | XVIII | XIX | XX | XXI |
| B-1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B-2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B-3 | 20 | 13 | 17 | 20 | 20 | 22 | 22 | 13 | 17 | 22 | 22 | 24 | 24 | 27 | 27 | 21 | 23 |
| B-4 | 9 | 10 | 8 | 8 | 9 | 8 | 8 | 8 | 8 | 9 | 9 | 10 | 9 | - | 9 | 8 | 8.6 |
| B-5 | 12 | 11 | 12 | 10 | 9 | 13 | 11 | 12 | 10 | 9 | 9 | 8 | 8 | 9 | 13 | 11 | 10 |

**Table 5:** Antibacterial susceptibility test of the isolates against Selected Chemical Compounds by Kirbey Bauer method performed in duplicate

| Chemical agent | Isolate no and zone of inhibition (mm) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV | XVI | XVII | XVIII | XIX | XX | XXI |
| C-1 | 12 | 13 | 8 | 9 | 8 | 9 | 13 | 10 | 8 | 10 | 11 | 13 | 9 | 8 | 12 | 11 | |
| C-2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C-3 | 8 | 8 | 9 | 8 | - | - | 8 | 9 | 7 | - | - | 8 | 8 | - | 8 | 8 | 8 |
| C-4 | 10 | 10 | 12 | 11 | 12 | + | 12 | 13 | 10 | 8 | 11 | 12 | 12 | 12 | 12 | 8 | 9 |

**Table 6:** Antibacterial susceptibility test of the isolates against Selected Microbial synthesized substances by Kirbey Bauer method performed in duplicate

| Microbial agent | Isolate no and zone of inhibition (mm) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV | XVI | XVII | XVIII | XIX | XX | XXI |
| M-1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M-2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| M-3 | 15 | 16 | 15 | 14 | 16 | 14 | 15 | 16 | 13 | 14 | 13 | 15 | 13 | 16 | 12 | 12 | 12 |
| M-4 | - | - | - | - | - | - | - | + | + | - | - | + | + | - | + | + | + |
| M-5 | 10 | 9 | 9 | 8 | 9 | 10 | 10 | 10 | 9 | 9 | 10 | 9 | 10 | 9 | 10 | 8 | 8 |

From the above results it has been observed that from each one of the originated substance only few can give the antibacterial activity and hence they are as follows.

**Table 7:** Extracts selected for further tests are with best results

| Botanical extract | | | Chemical | Microbial |
|---|---|---|---|---|
| Cold Methanol extract Hibiscus brackwnridgei (Yellow Hibiscus) flower | Dr. Batra's Hand sanitizer | Cold Acetone extract Hibiscus brackwnridgei (Yellow Hibiscus) flower | Alcohol | Cell free extract (Supernant) of Lactobacillus isolated from Idli batter |

### 3.6 Contact Inhibition assay

As from the above chart it has been observed that the antimicrobial agents used out of which only few have shown satisfactory results. Hence with these results the further study has been conducted. The results are as follows where it has been shown that minimal contact of agent with organism can inhibit growth for certain substances.

**Table 8:** Contact time study effect

| Antibacterial agents | Isolate no | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV | XVI | XVII | XVIII | XIX | XX | XXI |
| B-3 | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr |
| C-4 | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr |
| B-5 | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr |
| C-1 | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr | > 1 hr |
| M-2 | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr | >3 hr |

### 3.7 *In Vivo* study of pomegranate infection

The pomegranate fruits being infected by inoculation of isolates obtained out of all 6 have selected which have been swabbed on the surface of the pomegranate. On 24 hrs. of incubation no changes in the fruit observed as the days progressed the changes being observed in which a term day 2 the development of fungi observed. The fungi is yellow coloured and have completely infected the fruit. No blackening or lesions or cracks being observed. Hence no bacterial infection the observed is only for fungal growth.

Pomegranate after 1 week of incubation after inoculation and spraying

| Antibacterial agent | 0 weeks | 1 week | 2 weeks | 3 weeks | 4 weeks | Inference |
|---|---|---|---|---|---|---|
| Control | No change | No change | Fungal growth observed | Complete fungal mycelial covered | Complete fungal mycelial covered | No infection observed by isolate |
| B-3 | No change | No change | Fungal growth observed | Fungal growth | Complete fungal mycelial covered | No infection observed by isolate |
| C-4 | No change | No change | Fungal growth observed | Fungal growth | Complete fungal mycelial covered | No infection observed by isolate |
| B-5 | No change | No change | Fungal growth observed | Fungal growth | Complete fungal mycelial covered | No infection observed by isolate |
| C-1 | No change | No change | Fungal growth observed | Fungal growth | Complete fungal mycelial on covered | No infection observed by isolate |
| M-2 | No change | Fungal growth initiated | Fungal growth observed | Fungal growth | Complete fungal mycelial on covered | No infection observed by isolate |

## 4. Discussion

As all the phytopathogen are mainly observed to be viruses and fungi. On the major group of plant crops viral disease are being observed instead on pomegranate the fungal and bacterial infections are observed to be very prone and no viral disease. The fungal control can be controlled by using various control measures including traditional as well as modern fertilisers and antifungal agents. Thus it can be prevented for the infections from fungi. In case of the current study conducted *Xanthomonas* is a Gram negative rods, aerobic and catalase producer. (Patil A. G., *et al*., 2017) [1] The phylogenetic tree for this spp is very wide and almost all the bacteria belongs to phytopathogen groups majorly. As no chemical or traditional methods are being effective against this organism. As no control measure being observed which can be due to immunoreceptors for Xap is enough variable. Also the genetic variation from geological locations. The difference in Xap 's genetic variation leading to difficulties in control measures.

This study also shown that the botanical extracts out of which the *Hibiscus backwnrigie* flower with cold Methanol extract is observed to be highly effective and Dr. Batra'a hand sanitizer has also shown the satisfactory results. Along with Methanol extract Acetone extract observed to be effective. As Methanol extract observed to show the higher zones of inhibition against each organism the zone size ranges from 20-27 mm,

which is quite good results and is a broad range antibacterial agent which can control *Xanthomonas spp*. No effect has be seen given by Neem extract. As *Xanthomonas* can also be controlled by Dr. Batra'a hand sanitizer as is a Tulsi based hand sanitizer has been used as a control to detect whether is effective against broad range of organisms. Along with Methanol Acetone solvent based extraction shown to be effective but not as much as Methanol extract. As in case of other botanical extrcats used as A

Also chemical compounds being under study only Alcohol 70% which is used for regular lab works helps in major control of *Xanthomonas*. It has been checked as increased alcohol tolerance by many of organisms. Hence this indicates that the alcohol can be sprayed for control of blight disease. As in the rural areas the fruit waste or food waste observed to be used for alcohol production and thus can be cost effective. Thus can be studying large scale by producing the alcohol by using fruit waste so that can lead to give the better results. Another compound that is Streptocycline at these concentration have been used for study, as is a Streptomyces byproduct and thus can be effective and product in is also cost effective, but alone for better results require higher concentration thus for lower concentration to be used one can go for use in combination with other compounds as Copper oxychloride, etc. The effect of these can be studied further. The sulphur is an another compound that has been studied as no control observed indicates that it does not have activity against bacteria and effective against fungi. But the alcohol can lead to moisture and thus can make it prone to infections by fungi and hence can be used in combination and study it's activity against both fungi and bacterial.

Th Microbial substances that has been studied involves the bactericidal produced by a Bacillus spp. The *Lactobacillus spp* can produce bacteriocin which have shown better results which might be effective against isolates. Thus can be used for control. As no much effect being observed by other extracts inclusive of Pyocyanin. Hence the specific organisms can show inhibitory effect against the isolated.

The time of exposure to the isolate can lead to show the effectiveness of the compounds against isolate within shorter period time. As Hibiscus extract, alcohol, Dr Batra'a hand sanitizer and Streptocycline have shown the activity within less than 1 hr, but more than 30 mins of contact. Thus this can say that the increased time of exposure can lead to increased activity hence can be confirmed by performing colony forming unit assay and can study the decrease in loads. Also the decrease in load can be observed due to Lactobacillus extract which can serve as crude bacteriocin and hence is much effective against isolates with contact for more than 3 hrs, which is very longer period of exposure, hence cannot be effective criteria but if shows complete that is no single organism left can increase its applicability. Also no change in the quality parameters should be expected and hence need to be checked after completion.

*Streptomonas maltophilia* dominant member of Rhizosphere of microbial community and a plant root associated bacterium also produce high amounts of plant growth hormone indole-3-acetic acid. Using PCR for distinguishing *Xanthomonas* from the isolate are a phytopathogen. This phytopathogen being also involved in human infections. Hence may lead to cause mainly secondary infections in human. The control measures for both these isolate that is from soil and human infection have similarity only control measures vary due to difference in cell wall content and other characteristics. Also only single spp of Streptomonas being knownand have properties as that same of Xanthomonas only differ in certain natures as utilizes only 23 nutrient out of 145 organic nutrients and methionin is used. Also have more than one flagella and produce fimbriae as well.

The another identified organism is *Spinhomonas paucimobilis* which is a commonly isoalte obtsined from soil and does not belong to phytopathogen group and also not involved in human pathogen strain. Thus successfully can be used for bioremediation against PHA and can control pollution due to these highly complex cpmpounds.

The another isolate *Pantoae* spp does not belong to similar dendogram and have different branch. No connection with phytopathogen but associated with human coliform pathogens. Hence this may lead to cause infection in human due to ingestion of this pathogen.

Thus molecular techniques can be used for idnetification for further detection.

## 5. Conclusion

As our study suggests that the simple and cost effetive meaures can be identified by using different extracts from botanical, chemical and Microbial origin.

The isolate obtained can be *Xanthomonas* spp or as identified organisms which are different than phytopathogen. This might be due to poor hygiene conditions maintained or else the contaminated water supply or the human originated fertiliser being used might have given the presence of these pathogens. Hence need to re isolate the organisms. Also can VITEK or perform biochemical for remaining isolates. And also can use Molecular detection for identification.

The effectiveness of compounds being studied hae shown satisfactory results which indicates the selective activity of the specific compounds on organisms. Hence need to check for its activity on standard organisms isolated for the same spp.

The exposure time assay have given that majorly botanical extract being highly effective as well as chemical originated substances and thus can rely on theses results. Also the usage of this have shown the bacterial control but no effect on fungi hence need to look for combinations by which bot can be controlled. Also the micros extract can be used for nanoparticles synthesis may give better results.

Along with usage of these compounds one should check for the producivity does not hamper. Also the quality of the fruit must not degrade. Thus need to check for quality.

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