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MANAGEMENT OF BACTERIAL CANKER OF CARICA PAPAYA L. CAUSED BY ERWINIA SPECIES USING SOME HERBAL EXTRACTS.

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ABSTRACT:

The significant decline in papaya yield is attributed to the widespread occurrence of bacterial canker disease, which is caused by *Erwinia* species affiliated with the Enterobacteriaceae family and closely linked to *carotovora, crysanthemi*, as well as *cypripedii* species. Manifested by water-soaked angular lesions on leaves and stems, bacterial canker is a notable feature. These pathogens, which are temporary inhabitants of the soil, exhibit resilience even at temperatures as low as 28°C. Given the imperative of maximizing yield, effective control measures against the disease are essential. Conventional treatments such as bactericides, antibiotics, and fluorescent pseudomonades have proven ineffective in managing papaya bacterial canker. Consequently, the present investigation employs the well diffusion technique to evaluate the antibacterial properties of leaf and stem extracts derived from *Annona squamosa*, *Punica grantum*, *Citrus reticulata, Psidium guajava,* and *Manilkara zapota*, extracted in methanol, 50% ethanol, and methanol+chloroform solutions, against bacterial canker. The leaf and stem extracts exhibited substantial growth inhibition zones against *Erwinia* spp. Given that the selected herbs are widely cultivated cash crops and potential barriers to *Erwinia* spp. transmission, they offer the prospect of establishing multi-story tree cropping systems that not only enhance economic returns but also contribute to the ecological equilibrium of the environment and optimize the utilization of natural resources available.

Keywords: - *Carica papaya L. Bacterial canker, Erwinia spp.* Herbal extracts, Multi-storey tree cropping

INTRODUCTION:

Papaya's origin is believed to be in Southern Mexico and Costa Rica, and it was subsequently cultivated as a plantation crop in various countries such as Australia, Hawaii, and India for both commercial and home garden purposes (Bruneton 1999). Due to its polygamous nature, distinguishing between male, female, or hermaphrodite papaya plants can be challenging. The papaya tree typically reaches a height of 3 to 10 meters, displaying a palm-like appearance with a fleshy stem bearing scars from shed leaves, and a terminal cluster of leaves with 5-7 lobes on lengthy petioles. The fragrant and trimerous flowers of the papaya are predominantly unisexual dioecious, with male flowers in lax, densely pubescent clusters and female flowers on pendulous, fistular rachises, along with solitary large clusters of flowers on short, thick rachis. The fruit of the papaya is a large berry that comes in varying shapes from elongated to globose, featuring a central cavity of varying sizes, containing black, tuberculous seeds. These fruit-bearing trees reach maturity in just over a year, with milky liquid found in their leaves and immature fruits. The papaya, scientifically known as *Carica papaya*, plays a significant role as a tropical cash crop with considerable economic

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value for export, especially in small agricultural systems globally.

In the early 20th century, a disease caused by *'Bacillus papaya'* was identified in Java, later classified as *Erwinia herbicola*, leading to a purple discoloration on *Carica papaya* plants (Rant 1931; Magrou 1937; Nelson and Alvarez 1980). Reports of bacterial decline affecting papaya in the Mariana Islands due to two Erwinia species were recently documented (Trujillo and Scroth 1982), while similar occurrences were also reported in the US Virgin Islands, Venezuela, and the French West Indies, termed "bacterial stem canker" (Webb 1985; Guevara *et al.* 1993; Frossard *et al.* 1985). Symptoms of bacterial stem cankers on Solo-type papaya cultivars include greasy, water-soaked lesions on leaves progressing to angular foliar lesions, and the development of firm water-soaked cankers on the stem, often resulting in tree mortality (Prior *et al.* 1985; Webb 1985). The pathogen causing these cankers led to the rapid death of infected trees, with a survival period of up to two weeks in soil but indefinitely in leaf lesions or cankers, as well as on non-host leaves as an epiphyte. Disease severity and pathogen survival on leaf surfaces are not impacted by free moisture, with greasy, water-soaked spots on the skin being common symptoms of bacterial canker, caused by *Erwinia spp.* bacteria. Bacterial canker is primarily attributed to *Erwinia* spp. like Erwinia carotovora, *Erwinia chrysanthemi*, and *Erwinia cypripedii*, known for causing bacterial cancer (Webb 1985).

E. carotovora a plant pathogen belonging to the Enterobacteriaceae family, is a rod-shaped Gram-negative bacterium that lacks spores and exhibits peritrichous flagellation. This anaerobic facultative anaerobe causes cell death by inducing osmotic fragility, facilitated by the secretion of extracellular pectic enzymes that break down pectin integrity and the production of extracellular cellulase for cellulose degradation. Initially classified within Pectobacterium alongside other pectolytic phytopathogenic Erwinia species, *E. carotovora* displays diverse transmission modes among plants, predominantly through aerosol infection. Recent findings have identified the presence of *E. carotovora* in water sources worldwide, suggesting entry into water reservoirs via aerosols and runoff, with survival in soil remaining uncertain. Taxonomic updates have renamed *Erwinia chrysanthemi* as *Dickeya dadantii* (Grenier 2006).

Dickeya dadantii, a Gram-negative bacillus within the Enterobacteriaceae family, is known for fermenting carbohydrates to lactic acid as a facultative anaerobe, with most family members being plant pathogens. This straight rod-shaped, motile cell with rounded ends ranges in size and is peritrichous, surrounded by flagella (Nelson *et al.* 2009). *D. dadantii* induces plant diseases like necrosis, blight, and "soft rot," involving tissue maceration through pectinases that break down plant cell walls, leading to nutrient release for bacterial growth (Vaerenbergh *et al.* 2012). Commonly affected plants include ornamentals, vegetable bulbs, and potato tubers, with water splashing, insects, and cultural practices aiding in pathogen spread. Insects serve as crucial vectors for disease dissemination, carrying the bacteria externally and internally without harm.

Efforts to control the illness caused by *Dickeya dadantii* using commercial bactericides, antibiotics, and hostile fluorescent pseudomonas have proven ineffective, with resistance observed in certain land cultivars from the Virgin Islands and the Eastern Caribbean. Conversely, commercial cultivars from various regions, such as Jamaica, Costa Rica, Hawaii, and Puerto Rico, exhibit high susceptibility to bacterial canker (Webb1985). Limited treatment options exist due

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to the time-sensitive nature of bacterial canker management.

Pulses are a valuable source of protein, vitamins, and minerals. Pulses by-products are fed to livestock as a dry and fresh feed. In Asia, Africa, and the Caribbean, *Lablab purpureus* is produced as a pulse crop.

MATERIALS AND METHODS:

All the chemicals and reagents used were from LOBA chemie Pvt. Ltd, Mumbai, India, Sigma Chemical Company, Louis, Oxoid Ltd., London, Research Lab. fine Chemical industries Mumbai, India. The media and broth used for microbial culture were from Hi-Media Pvt. Ltd, Bombay, India.

Sample collection

Samples of the potentially pathogenic leaves and stems were taken from various parts of Maharashtra (India), including Pawnar, Seloo of Wardha district, Nerpinglai Morshi of Amravati district, Katol of Nagpur district, Gahuli, Pusad, and Mahagaon of Yavatmal district, Mehkar of Buldhana district, Malegaon of Nashik district Risod of Washim district, and Patur of Akola district and Warora of Chandrapur district. The whole plant was refluxed in running tap water for 1-2 hrs and sterilized by 0.1% (w/v) $HgCl_2$ with two drops of Tween 80 for 2 min. followed by rinsing with sterile distilled water until all traces of sterilant are removed (Gawde and Paratkar 2004)

Herbal plants selected for the study.

The fresh leaves and stems of plants such as *Annona squamosa, Citrus reticulata, Punica granatum, Psidium guajava, Manilkara zapota* leaves and stems were collected from the farm of Inzapur, Wardha (MS) and authenticated by Prof. Dr. C.B. Shende, Department of Biotechnology and Plant tissue culture NACSC Wardha (MS). Herbarium of same were prepared and kept in department.

Isolation of *Erwinia spp.*

Possible diseased leaves and stems were surface sterilized, washed aseptically and teased with sterilized razor and again surface sterilized in 10% Sodium hypocloride solution for 1 min, further washed with distilled water for 3-4 times and 100µl of suspention pour on plates containing King's medium B and allowed to incubate at 28 ^{0}C for 24-48 hrs and different species were characterized by those standard determinative morphological observations and physiological tests (Bergey 1994; Schrothand Hilderbr and 1983; Lelliotand Stead 1987). King's medium B and slant are used for maintaining pure culture of *Erwinia species* preserved in freezer at 4 ^{0}C for further use (King *et al.* 1954)

Preparation of bioactive plant extracts

Sample preparation was of utmost importance to the development of analytical methods for the analysis of constituents present in the botanicals and herbal preparations. A typical extraction process involves collection and authentication of plant material & drying, size reduction, extraction, filtration, concentration, drying & reconstitution. Quality of an extract was influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material, solvent ratio etc. From laboratory scale to pilot scale all the parameters are optimized and controlled during extraction. Extraction techniques separated the soluble plant metabolites through selective use of solvents (Handa *et al.* 2008).

Extracts of *Anonna squamosa, Punica granatum, Citrus reticulata, Psidium guajava, Manilkara zapota*

The leaves and stems were collected washed, shade dried and about 100 gm leaves weighed and powdered. The powdered form of these leaves and stem were further extracted with absolute methanol, 50% ethanol and methanol+chloroform for overnight at 37 ^{0}C. This

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extract was further filtered with filter paper and concentrated by keeping in hot air oven to get a dark brown semi-solid residue, which was then weight and the percentage of recovery of extract was noted (Mathew *et al.* 2002) One portion of residue was then weighted and restituted in same solvent and kept as stock solution for invitro evaluation against *Erwinia spp.* by following a slight modified method (Ghosh *et al.* 1993)

Screening of herbal plant extract against *Erwinia spp.*

Leaves and stems extracts were prepared in different solvents and screened for anti-Erwinial activity by well diffusion method in which the various extracts were diffused through the solidified King's medium so, that the growth of microorganisms is inhibited in a circular area or zone around the wells containing extracts (Toit and Rautenbach 2000; Bagamboula *et al. 2004*; Raju *et al. 2011*). The cup-plate agar diffusion method was employed to assess the antibacterial activity of the prepared extracts (Popoola *et al. 2007)*20 ml of the inoculated medium was distributed into sterile Petri dishes. The agar was left to set and in each of these plates, 5 mm in diameter, was cut using a sterile cork borer No. 4 and the agar discs were removed (McCarrell *et al. 2007)*. Alternate cups were filled with 20 µL of each extract using a microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 28^0 C for 18 hours. The respective solvents were used as controls. The diameters of the growth inhibition zones were measured at 24 hours of incubation averaged and values were tabulated.

RESULT & DISCUSSION:

The current investigation is focused on mitigating bacterial canker in *Carica papaya* by employing extracts derived from the stems and foliage of various herbs, which have been processed in methanol, 50% ethanol, and a blend of methanol and chloroform, through the well diffusion technique. The subsequent findings and outcomes exhibit encouraging prospects.

Zone of inhibition (In mm) of *Erwinia spp.* when screened with different leaves extracts prepared in methanol

Punica granatum exhibits the strongest antibacterial activity among the methanolic extracts of the plants mentioned above, whereas *Annona squamosa* is weak against *E. carotovora. Psidium guajava, Manilkara zapota*, and *Citrus reticulata* are reported to have modest effects. While *Annona squamosa* is proven to be neutral, Manilkara zapota extract has the highest antibacterial activity against *E. crysanthemi*. Transitional substances include *Punica granatum, Psidium guajava*, and *Citrus reticulata. E. cypripedii* exhibits a moderately suppressed response to *Citrus reticulata*, *Psidium guajava*, and *Punica granatum*, and is completely indifferent to *Manilkara zapota* and *Annona squamosa*.

Zone of inhibition (In mm) of *Erwinia spp.* when screened with different leaves extracts prepared in 50% Ethanol.

It was observed that the 50% ethanolic extract had low antibacterial action. When tested against *E. carotovora, Psidium guajava* and *Punica granatum* extracts were relatively similar, whereas *Manilkara Zapota* extract is less noticeable but still exhibits some antibacterial action. *E. carotovora* becomes resistant to *Annona squamosa* and *Citrus reticulata* extract. Only *Psidium guajava* exhibits antibacterial action against *E. crysanthemi*. The extracts mentioned above, when produced in 50% ethanol, had no effect on *E. cypripedii* in any way.

Zone of inhibition (In mm) of *Erwinia spp.* when screened with different leaves extracts prepared in methanol + chloroform.

Comparing *Punica granatum* leaf extract to all other extracts, it is discovered that *E. carotovora* is extremely sensitive to it. When tested for efficacy against *E. carotovora*, *Psidium*

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guajava extract was found to be inert. *Citrus reticulata, Manilkara zapota*, and *Annona squamosa* are intermediary in their activity against the same. Only the leaves of *Psidium guajava* and *Manilkara zapota* cause *E. chrysanthemi*to become sensitive; *Annona squamosa, Punica granatum*, and *Citrus reticulata* have no effect. *Punica granatum* leaves extract has a strong inhibitory effect on *E. cypripedii*, while *Annona squamosa, Citrus reticulata,* and *Manilkara zapota* extracts have a more muted effect and *Psidium guajava* leaves extract had no effect at all.

Zone of inhibition (In mm) of *Erwinia spp.* when screened with different stem extracts prepared in methanol.

E. carotovora reacts similarly to *Annona squamosa* and *Punica granatum* stem extracts, but *Citrus reticulata* stem extract has no effect on it at all, and *Psidium guajava* and *Manilkara zapota* stem extracts cause it to react in a middle-of-the-road manner. *Punica granatum* methanolic extract was found to be effective in stopping *E. chrysanthemi* proliferation. The antibacterial activity of extracts of *Annona squamosa* and *Manilkara zapota* against *E. chrysanthemi*is moderate. The stems of *Annona squamosa, Punika granatum*, *Citrus reticulate, Psidium guajava*, and *Manilkara zapota* all exhibit similar antibacterial activity against *E. cypripedii* and a zone of inhibition that is identical across the board.

Zone of inhibition (In mm) of *Erwinia spp.* when screened with different stem extracts prepared in 50% ethanol.

When tested against *E. carotovora*, a 50% ethanol-prepared extract of *Punica granatum* was shown to have the highest level of growth inhibition, whereas extracts of *Citrus reticulata* and *Manilkara zapota* completely rendered it inactive. *Citrus reticulata* extract has no effect on *E. chrysanthemi* and is significantly inhibited by *Annona squamosa* extract, however *E. chrysanthemi* exhibits a mixed reaction to *Punica granatum*, *Psidium guajava*, and *Manilkara zapota*. With regard to *E. cypripedii*, *Punica granatum* stem extract exhibits the strongest antibacterial activity. *Citrus reticulata* stem extract had no effect on *E. cypripedii*, although *Psidium guajava*, *Manilkara zapota*, and *Annona squamosa* have an intermediate effect.

Zone of inhibition (In mm) of *Erwinia spp.* when screened with different stem extracts prepared in methanol+chloroform

If treated with *Punica granatum*, *Psidium guajava*, and *Citrus reticulata* extracts, the growth of *E. carotovora* is mostly inhibited. *Citrus reticulata* and *Manilkara zapota* extracts have weaker effects. *Manilkara zapota* stem extract has no effect on *E. chrysanthemi* growth; *Punica granatum* and *Annona squamosa* stem extracts have a strong inhibitory effect; and *Citrus reticulata* and *Punica granatum* have medium sensitivity. The stem extract of *Annona squamosa* significantly slows the growth of *E. cypripedii*, but the stem extracts of *Manilkara zapota*, *Punica granatum*, *Citrus reticulata*, and *Psidium guajava* have weaker effects.

A bacterium called *Erwinia spp.*, which also causes the Mariana and Java papaya illnesses, is the cause of papaya bacterial canker. While showing similarities that are near to those of *Erwinia carotovora*, the St. Croix isolates differ from the Mariana isolates and other *Erwinia* groups due to a combination of physiological and biochemical traits (Trujillo *et al.*1982). The current study aims to determine the best antibacterial capability of several herbal extracts synthesized in various solvents for the treatment of *Erwinia spp.* species that cause bacterial canker. Maximum antibacterial advantages were obtained when *Punica granatum* leaf extract was produced in Methanol, 50% ethanol, and a mixture of methanol+chloroform and screened with *Erwinia carotovora*. When *Punica granatum*

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stem extracts were made in methanol and 50% ethanol, the same outcome was obtained against *E. carotovora*. In addition, methanol+chloroform leaf and stem extracts have the greatest potential for slowing *Erwinia chrysanthemi* growth. Additionally, it has been discovered that *Erwinia chrysanthemi* is extremely sensitive to methanolic extracts of *Manilkara zapota* and *Psidium guajava* leaf extracts. When the stem extract of *Annona squamosa* was produced in 50% ethanol and methanol+chloroform and tested against *Erwinia crysanthemi*, the results were favorable. When both methanol-only and methanol+chloroform-prepared *Punica granatum* stem extracts have the same anti-*E. chrysanthemi* effects.

When made and tested in methanol and methanol+chloroform, the leaves extract of *Manilkara zapota* and *Punica granatum* are extremely toxic to *E. cypripedii*. While the entire 50% ethanolic extract was discovered to be completely ineffective against *E. cypripedii*. Repeat the results in the case of the stem methanolic extracts of *Punica granatum* and *Annona squamosa*. *Citrus reticulata* was discovered to have very low antibacterial activity, even after extracts of its leaves and stem were made and tested in several solvents, including methanol, 50% ethanol, and methanol with chloroform.

The most promising cultivar to date is Barbados dwarf, which in field testing is frequently the only cultivar that remains standing after the first year of production. Currently, the most efficient method of controlling the bacterial canker is the adoption of resistant cultivars. The adoption of suitable barrier crops that do not sustain a disease population's epiphytic population is a further control method under investigation (Webb1985). A mixed crop of *Carica papaya* (Linn) with *Punica granatum, Psidium guajava, Annona squamosa*, and *Manilkara zapota* might prevent the potential transmission of *Erwinia spp.* and develop into a fruitful multistoried crop, even though the mode of transmission of the bacterial canker pathogen varied from plant to plant but; via aerosol is most common.

CONCLUSION:

In connection with papaya roots or decomposing diseased plant remains, the bacterial canker plant pathogen did not survive well, showing that it is a temporary soil inhibitor But it continued to thrive for a very long time in the cankers and leaf diseases of the damaged trees. The attempt to control this disease with bactericides, antibiotics, and locally isolated antagonistic pseudomonas was unsuccessful, according to the report. The best course of action, then, is to employ a good barrier crop, such as *Punica granatum, Psidium guajava, Annona squamosa*, or *Manilkara zapota*, to control or prevent the epiphytic population of pathogens that results in multistory cropping.

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Table No.1. Biochemical, physiological and cultural characteristics of *Erwinia* spp.

Characteristics	*E.carotovora*	*E .crysanthemi*	*E.cypripedii*
Growth at 36 ^{0}C	+	+	+
Pectate degradation	+	+	+
Gelatin liquification	+	-	+
Growth 5% at 36 ^{0}C	+	-	-
Sensitivity to erythromycin	-	+	-
Urease	-	-	-
Indol	-	-	-
Starch hydrolysis	-	-	-
Citrate test	+	+	+
Motility	+	+	+
Staining	+	+	+
Flagellation	+	+	+

Table No. 2. Zone of inhibition (In mm) of *Erwinia* spp. when screened with different leaves extracts prepared in methanol.

Species of *Erwinia*	*Annona squamosa*	*Punica granatum*	*Citrus reticulata*	*Psidium guajava*	*Manilkara Zapota*
E.carotovora	10	21	20	19	16
E.crysanthemi	0	12	10	15	17
E.cypripedii	0	14	10	14	15

Table No.3. Zone of inhibition (In mm) of *Erwinia* spp. when screened with different leaves extracts prepared in 50% ethanol

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Species of *Erwinia*	*Annona squamosa*	*Punica granatum*	*Citrus reticulata*	*Psidium guajava*	*Manilkara zapota*
E.carotovora	0	20	0	21	10
E.crysanthemi	0	0	0	10	0
E.cypripedii	0	0	0	0	0

Table No. 4. Zone of inhibition (In mm) of *Erwinia* spp. when screened with different leaves extracts prepared in Methanol+chloroform.

Species of *Erwinia*	*Annona squamosa*	*Punica granatum*	*Citrus reticulata*	*Psidium guajava*	*Manilkara zapota*
E.carotovora	15	15	0	10	11
E.crysanthemi	10	12	7	8	10
E.cypripedii	10	11	10	10	10

Table No. 5. Zone of inhibition (In mm) of *Erwinia* spp. when screened with different stems extracts prepared in Methanol

Species of *Erwinia*	*Annona squamosa*	*Punica granatum*	*Citrus reticulata*	*Psidium guajava*	*Manilkara zapota*
E.carotovora	10	25	20	0	14
E.crysanthemi	0	0	0	10	5
E.cypripedii	13	17	13	0	10

Table No. 6. Zone of inhibition (In mm) of *Erwinia* spp. when screened with different stem extracts prepared in 50% ethanol

Species of *Erwinia*	*Annona squamosa*	*Punica granatum*	*Citrus reticulata*	*Psidium guajava*	*Manilkara zapota*
E.carotovora	17	20	0	15	10
E.crysanthemi	15	12	0	13	10
E.cypripedii	13	17	0	13	12

Table No. 7. Zone of inhibition (In mm) of *Erwinia* spp. when screened with different stem extracts prepared in methanol+ chloroform.

Species of *Erwinia*	*Annona squamosa*	*Punica granatum*	*Citrus reticulata*	*Psidium guajava*	*Manilkara zapota*
E.carotovora	20	10	7	15	7
E.crysanthemi	10	11	8	7	0
E.cypripedii	12	6	10	10	5

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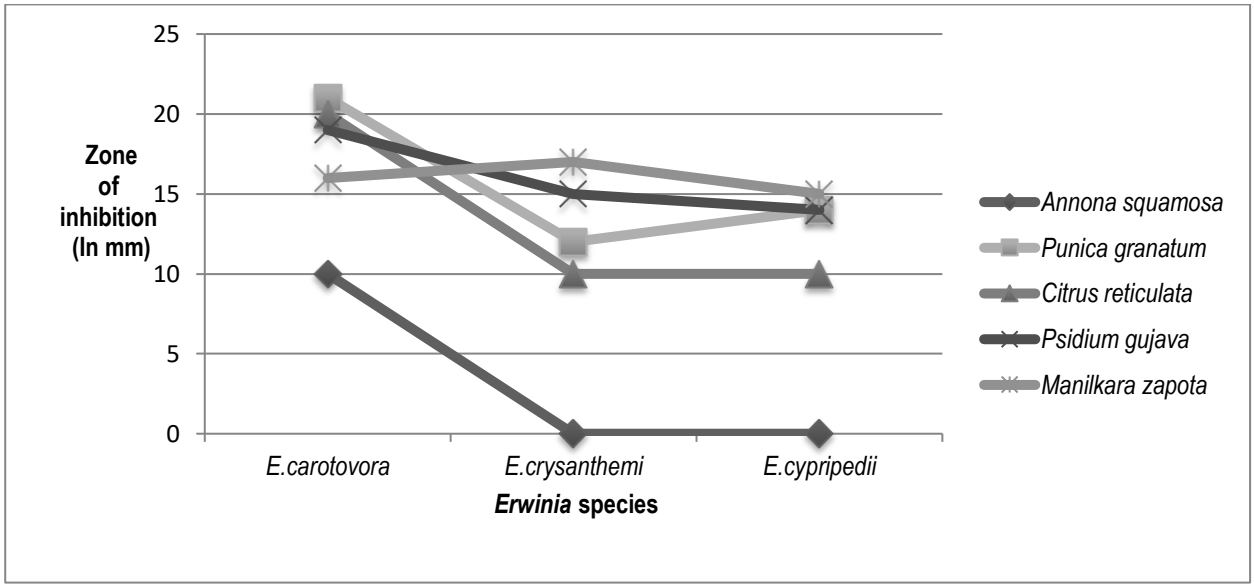
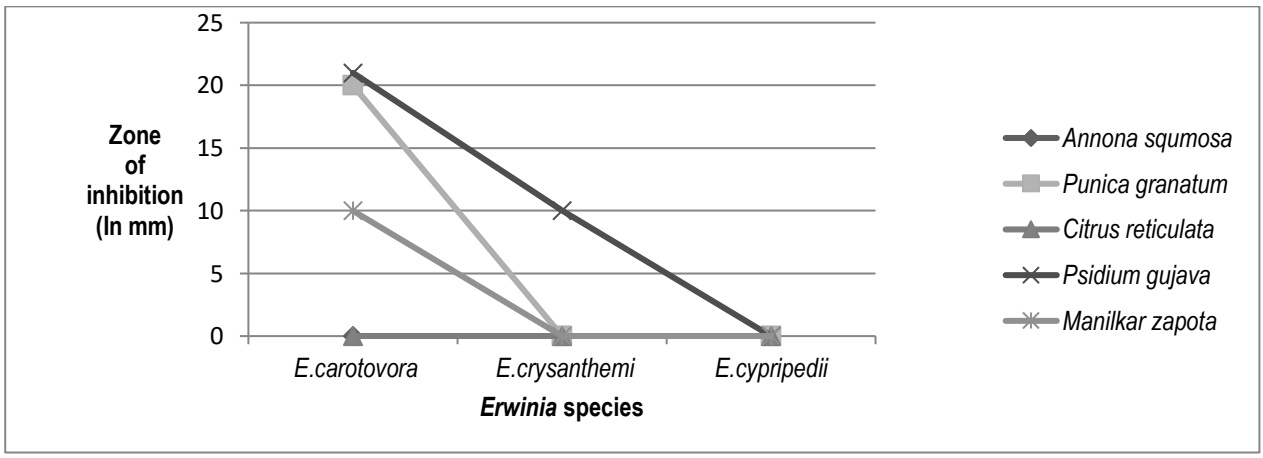
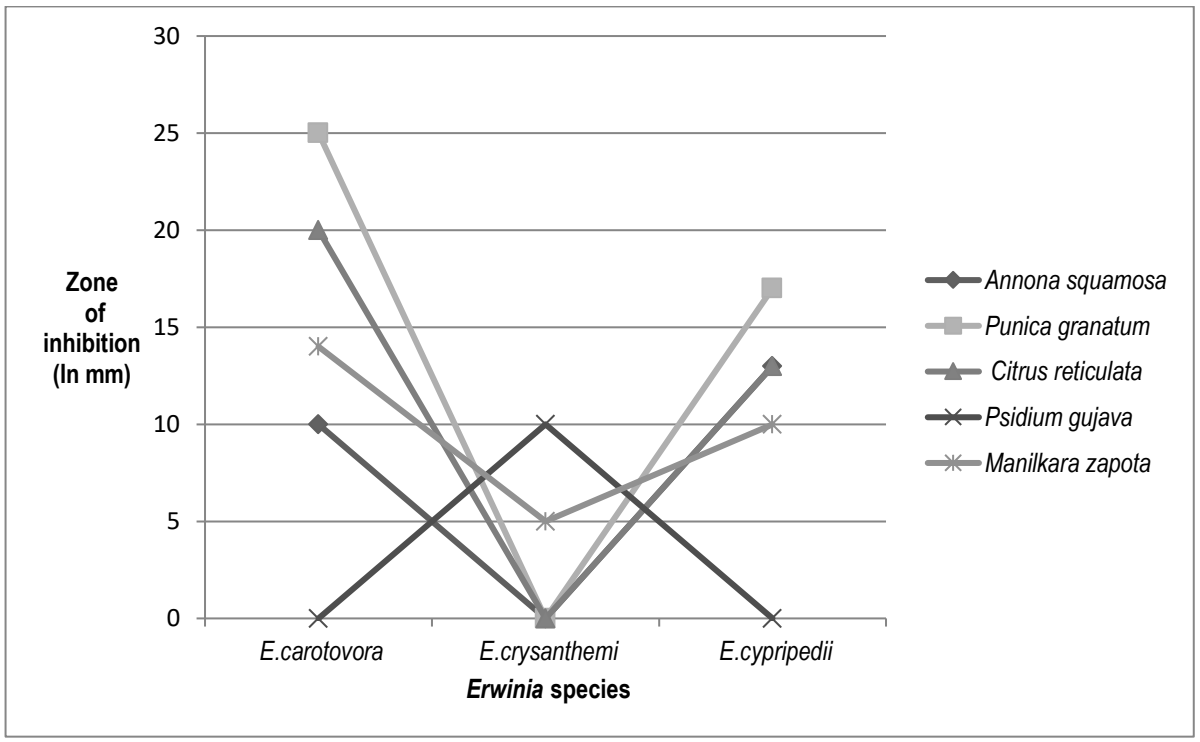


Graph 1. Zone of inhibition (In mm) of *Erwinia spp.* when screened with different leaves extracts prepared Methanol

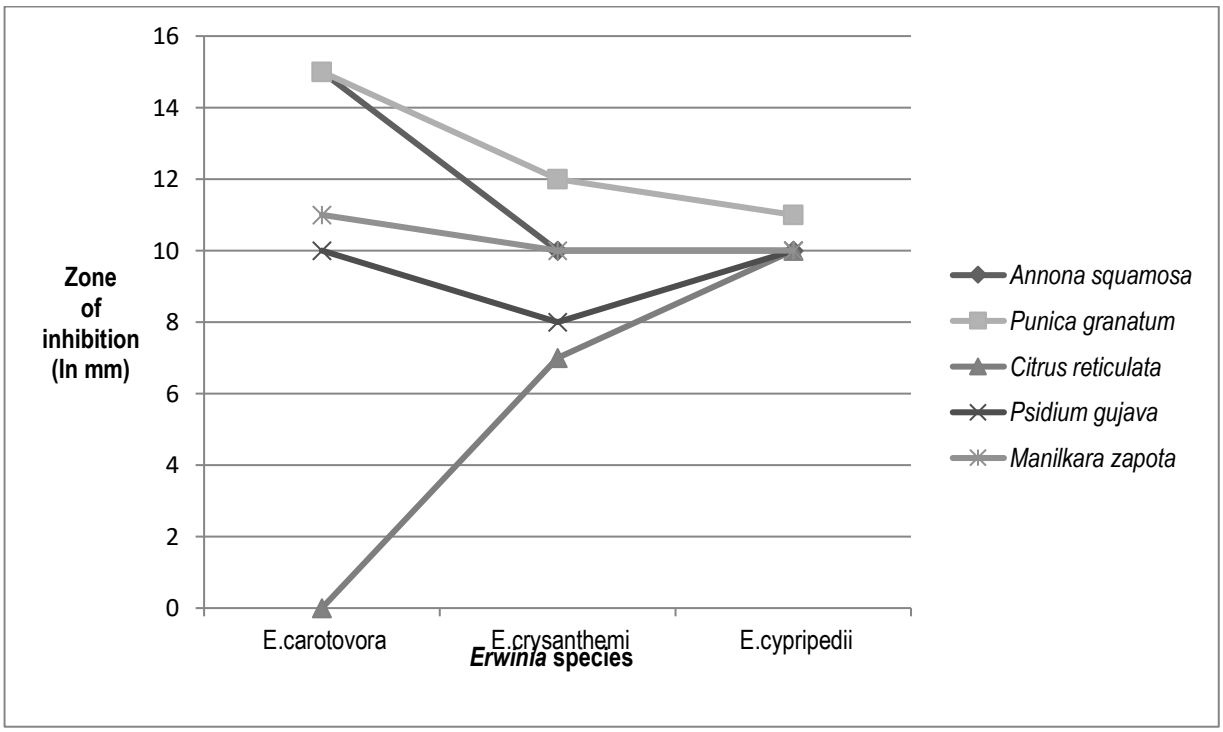


Graph 2. Zone of inhibition (In mm) of *Erwinia spp.* when screened with different leaves extracts prepared in 50% Ethanol

http://doi.org/10.29369/ijrbat.2025.010.1.0006

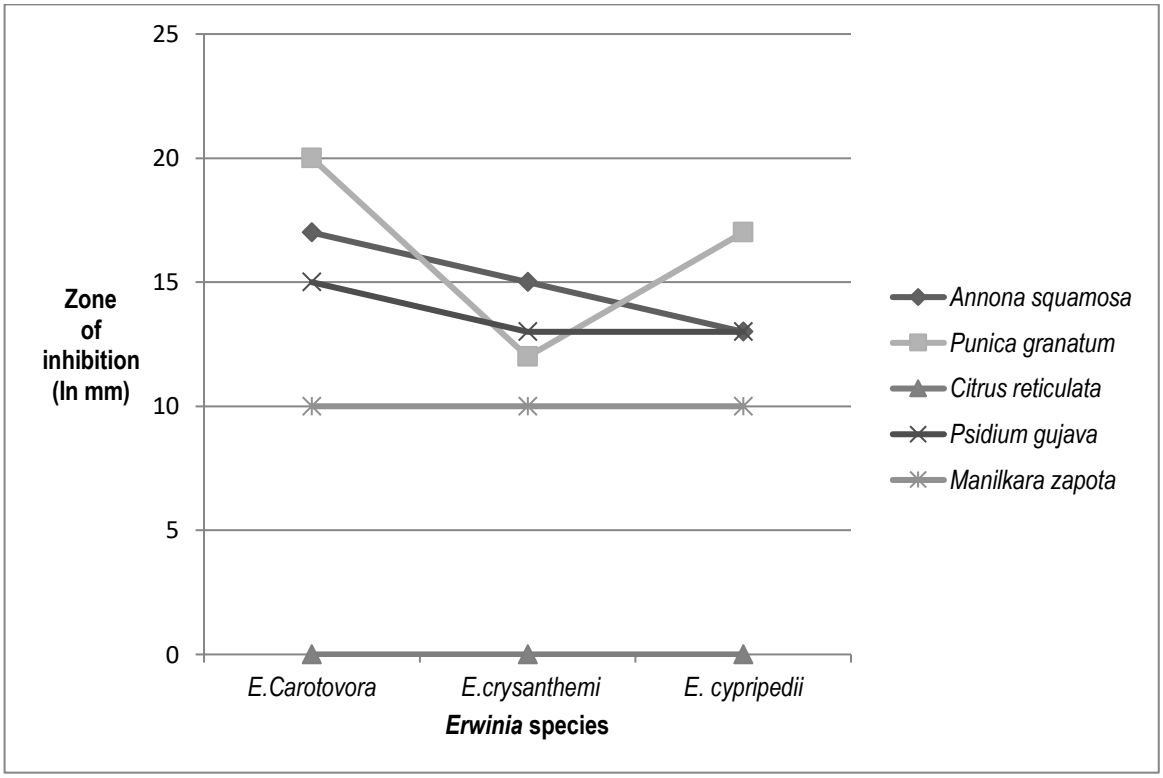


Graph 3. Zone of inhibition (In mm) *of Erwinia spp.* when screened with different leaves extracts prepared in Methanol+chloroform.

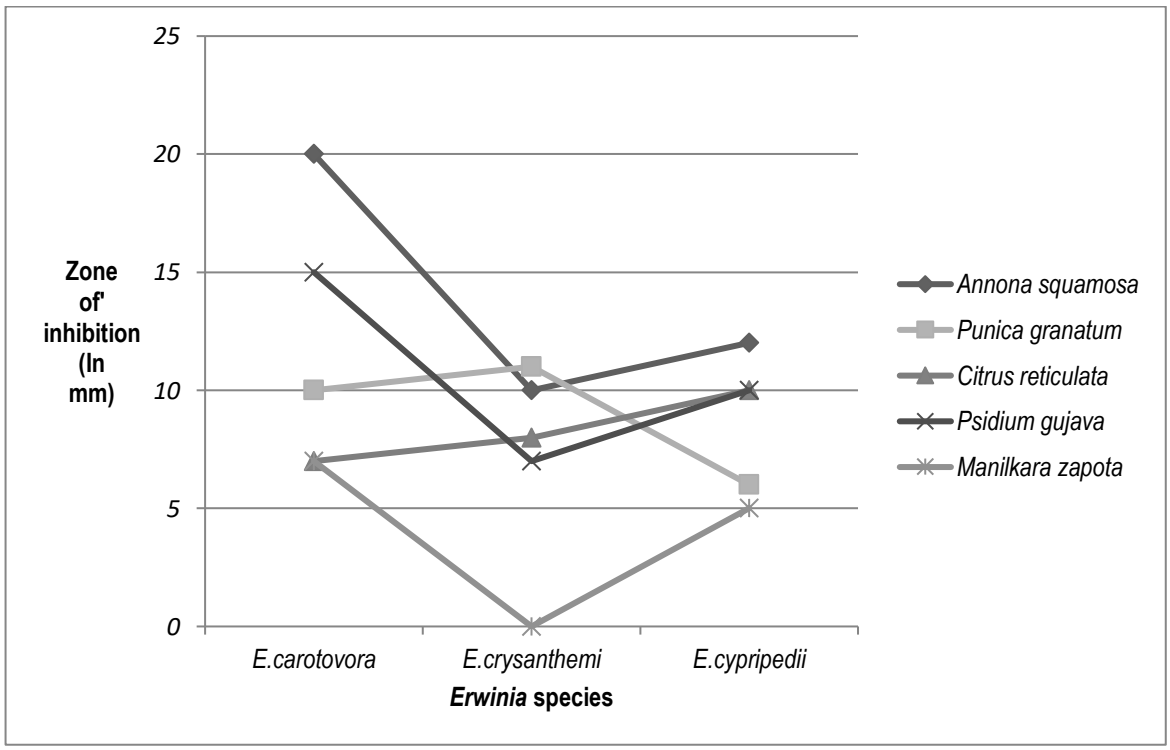


Graph 4. Zone of inhibition (In mm) of *Erwinia spp.* when screened with different stem extracts prepared in Methanol.

http://doi.org/10.29369/ijrbat.2025.010.1.0006



Graph 5. Zone of inhibition (In mm) of *Erwinia spp.* when screened with differentstem extracts prepared in 50% Ethanol.



Graph 6. Zone of inhibition (In mm) of *Erwinia spp.* when screened with different stem extracts prepared in Methanol+chloroform.

http://doi.org/10.29369/ijrbat.2025.010.1.0006

Fig 1. Infected leaf of Papaya plant

Fig 2. Infected stem of Papaya plant

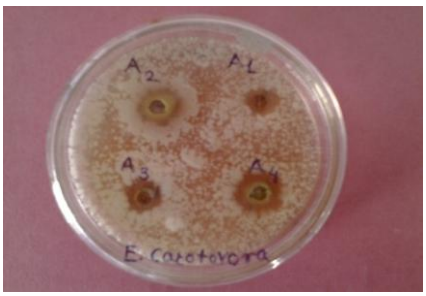


Plate 1 (Methanolic Extract of (A_1- *Annona squamosa* A_2 - *Punica grantum,* A_3 - *Citrus reticulata,* A_4 - *Manilkara Zapota)*

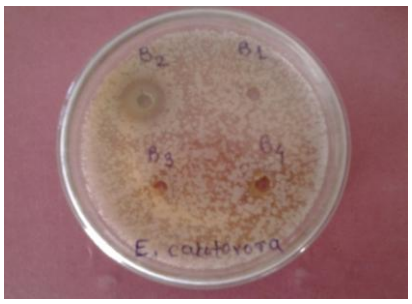


Plate 2 (Ethanolic Extract of (B_1- *Annona squamosa* B_2 -*Punica grantum,* B_3 - *Citrusreticulata,* B_4 -*Manilkara Zapota)*

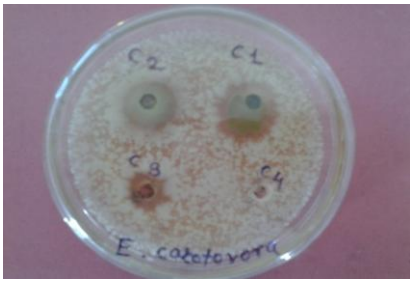


Plate 3 (Methanol+chloroform Extract of (C_1,- *Annona* squamosa, C_2 - *Punica grantum,* C_3 - *Citrus reticulata,* C_4 -*Manilkara Zapota)*

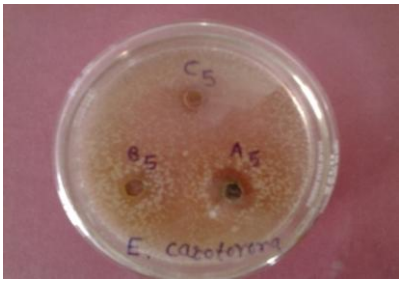


Plate 4 (Methanolic Extract of (A_5,- *Psidium gujava,* Ethanolic extract of B_5 -*Psidium gujava,Methanol+chloroform extract of* C_5 -*Psidium gujava)*

Plate 5 (A- Methanol, B- 50% ethanol ,C- Methnol+chloroform)

http://doi.org/10.29369/ijrbat.2025.010.1.0006

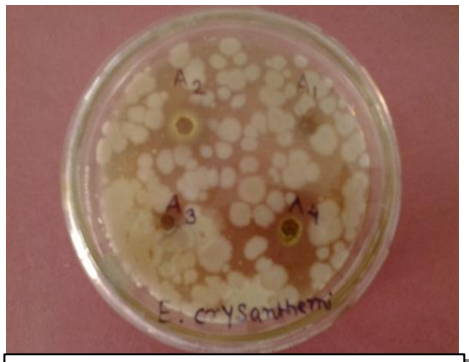


Plate 1 (Methanolic Extract of (A_1- *Annona squamosa* ,A_2 -*Punica grantum,* A_3 - *Citrus reticulata,* A_4 -*Manilkara Zapota)*

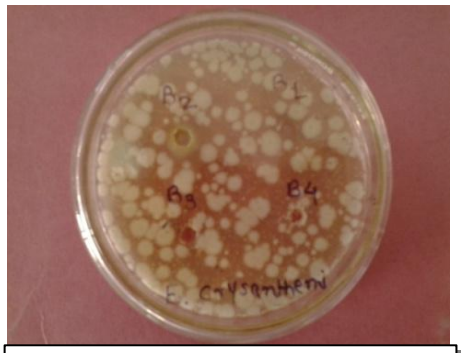


Plate 2 (Ethanolic Extract of (B_1,- *Annona squamosa,* , B_2 -*Punica grantum,* B_3 - *Citrus reticulata,* B_4 - *Manilkara Zapota)*

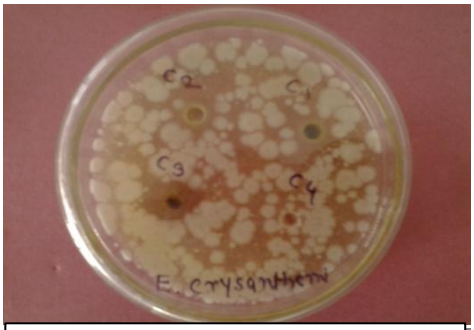


Plate 3(Methanol+chloroform Extract of (C_1,- *Annona* squamosa, C_2 - *Punica grantum,* C_3 - *Citrus reticulata,* C_4 - *Manilkara Zapota)*

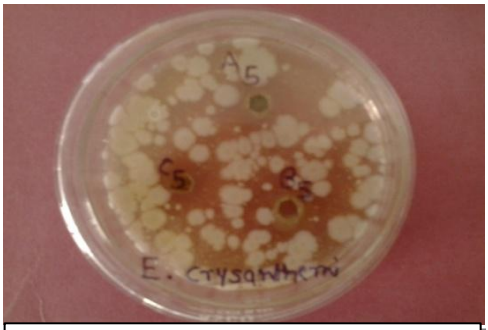


Plate 4 (Methanolic Extract of (A_5,- *Psidium gujava,* Ethanolic extract of B_5 - *Psidium gujava, Methnol+chloroform extract of* C_5 -*Psidium gujava)*

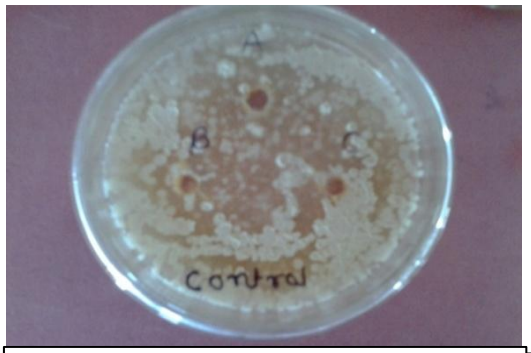


Plate 5 (A- Methanol, B- 50% ethanol ,C- Methanol+ choloroform)

Fig 4. Zone of inhibition (In mm) of *Erwinia spp*. When screened with different leaves extracts prepared in methanol, methanol, 50% ethanol, methanol+chloroform against *E.crysanthemi*

http://doi.org/10.29369/ijrbat.2025.010.1.0006

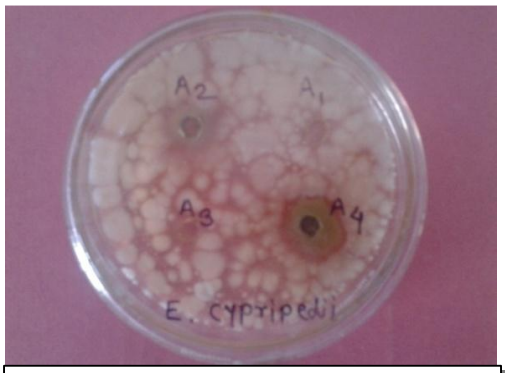


Plate 1 (Methanolic Extract of (A_1,- *Annona squamosa* A_2 - *Punica grantum,* A_3 - *Citrus reticulata,* A_4 -*Manilkara Zapota)*

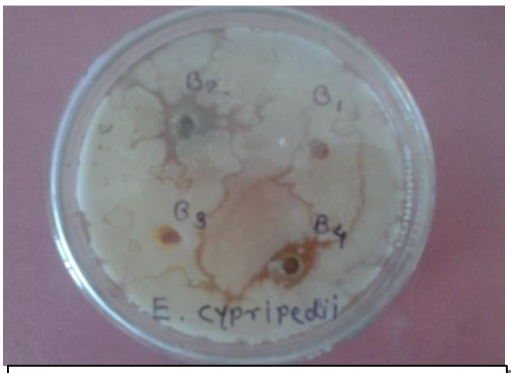


Plate 2 (Ethanolic Extract of (B_1,- *Annona squamosa* ,B_2 - *Punica grantum,* B_3 - *Citrus reticulata,* B_4 -*Manilkara Zapota)*

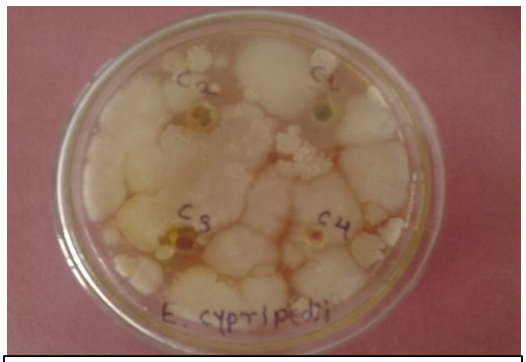


Plate 3 (Methanol+chloroform Extract of (C_1- *Annona* squamosa, C_2- *Punica grantum,* C_3 - *Citrus reticulata,* C_4 -*Manilkara Zapota)*

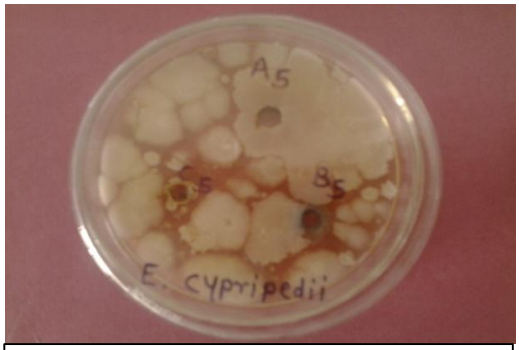


Plate 4 (Methanolic Extract of (A_5,- *Psidium gujava,* Ethanolic extract of B_5 - *Psidium gujava, Methanol+chloroform extrct of* C_5 - *Psidium gujava)*

Plate 5 (A-Methanol, B- 50% ethanol ,c- Methanol+ chloroform)

http://doi.org/10.29369/ijrbat.2025.010.1.0006

Fig 5. Zone of inhibition (In mm) of *Erwinia spp.* when screened with different leaves extracts prepared in methanol, 50% ethanol, methanol+chloroform against *Erwinia cypripedii.*

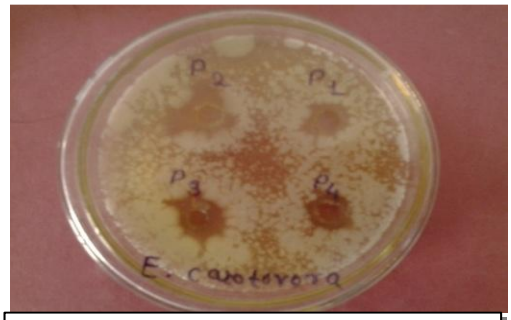


Plate 1(Methanolic Extract of (A_1,- *Annona squamosa* A_2 -*Punica grantum,* A_3 - *Citrus reticulata,* A_4 -*Manilkara Zapota)*

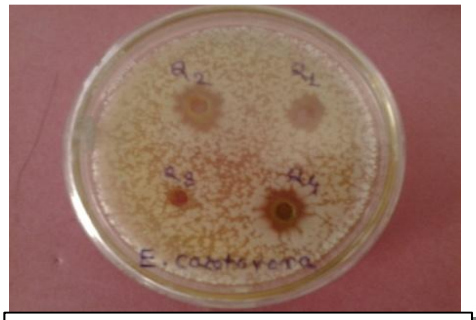


Plate 2(Ethanolic Extract of (B_1,- *Annona squamosa* B_2- *Punica grantum,* B_3 - *Citrus reticulata,* B_4 -*Manilkara Zapota)*

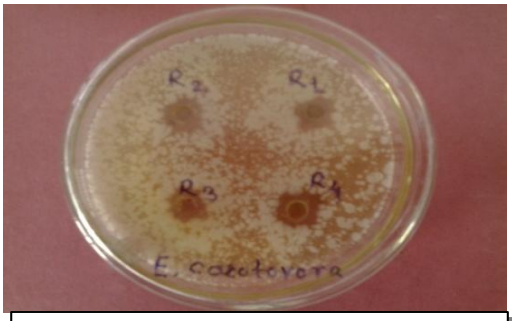


Plate 3(Methanol+chloroform Extract of (C_1,- *Annona* squamosa, C_2 - *Punica grantum,* C_3- *Citrus reticulata,* C_4 - *Manilkara Zapota)*

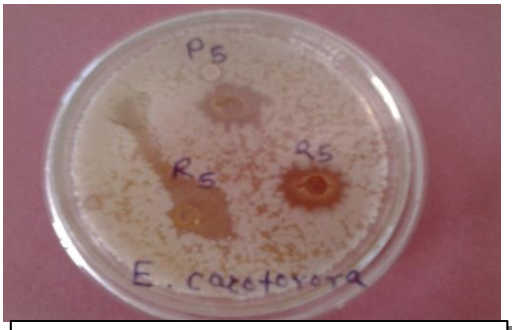


Plate 4(Methanolic Extract of (A_5- *Psidium gujava,* Ethanolic extract of B_5 - *Psidium gujava, Methnol+chloroform extract of* C_5 - *Psidium gujava)*

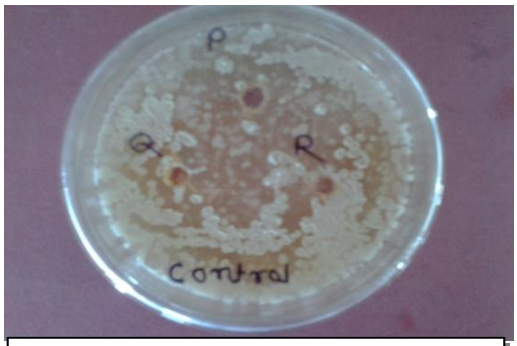


Plate 5 (P- Methanol, Q- 50% ethanol ,R- Methnol+ chloroform)

Fig 6. Zone of inhibition (In mm) of *Erwinia spp.* when screened with different stem extract prepared in methanol, 50% ethanol, methanol+chloroform against *E. carotovora.*

http://doi.org/10.29369/ijrbat.2025.010.1.0006

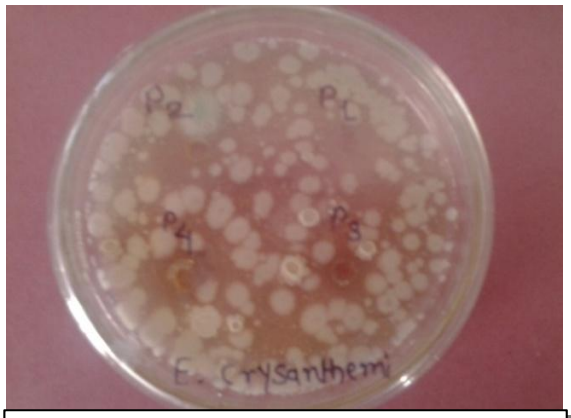


Plate 1 (Methanolic Extract of (A_1,- *Annona squamosa,* A_2 - *Punica grantum,* A_3 - *Citrus reticulata,* A_4 -*Manilkara Zapota)*

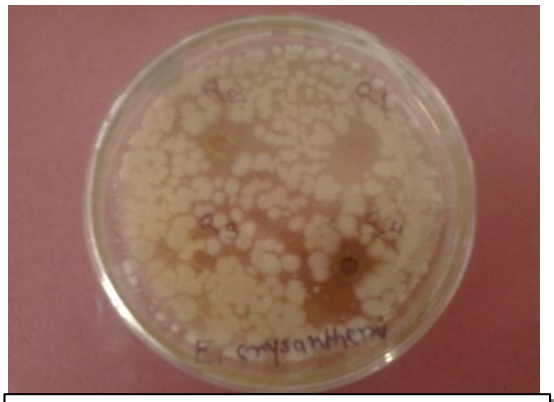


Plate 2 (Ethanolic Extract of (B_1,- *Annona squamosa* ,B_2 -*Punica grantum,* B_3 - *Citrusreticulata,* B_4 -*Manilkara Zapota)*

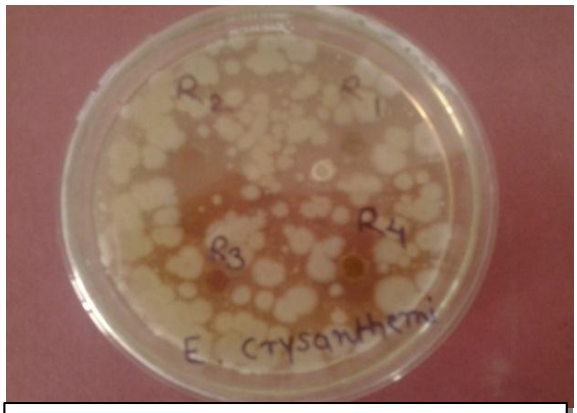


Plate 3 (Methanol+chloroform Extract of (C_1,- *Annona* squamosa, C_2- *Punica grantum,* C_3 - *Citrus reticulata,* C_4 -*Manilkara Zapota)*

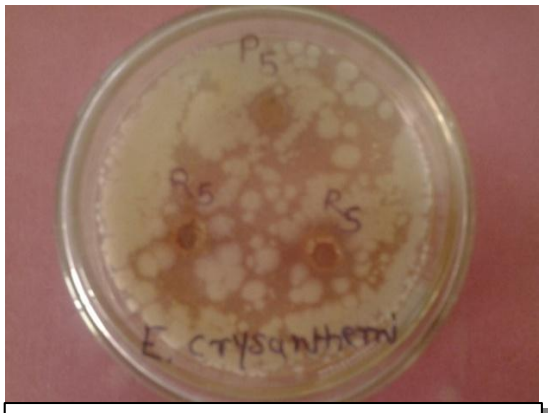


Plate 4 (Methanolic Extract of (A_5,- *Psidium gujava,* Ethanolic extract of B_5 - *Psidium gujava, Methnol+chloroform extract of* C_5 - *Psidium gujava)*

Plate 5 (P- Methanol, Q- 50% ethanol ,R- Methnol+ chloroform)

Fig 7. Zone of inhibition (In mm) of Erwinia spp. when screened with different stem extracts prepa red in methanol, 50% Ethanol, methanol+chloroform against *E.crysanthemi.*

http://doi.org/10.29369/ijrbat.2025.010.1.0006

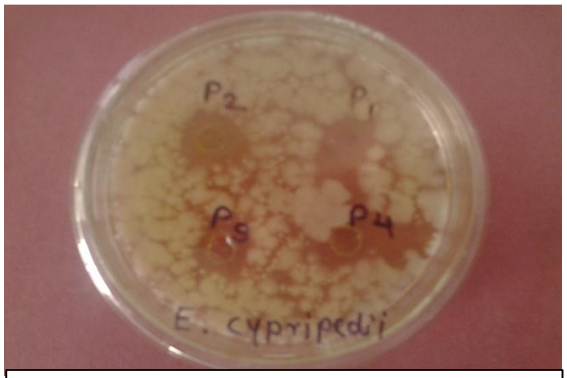


Plate 1 (Methanolic Extract of (A_1,- *Annona squamosa,* A_2 *-Punica grantum,* A_3 *- Citrus reticulata,* A_4 *-Manilkara Zapota)*

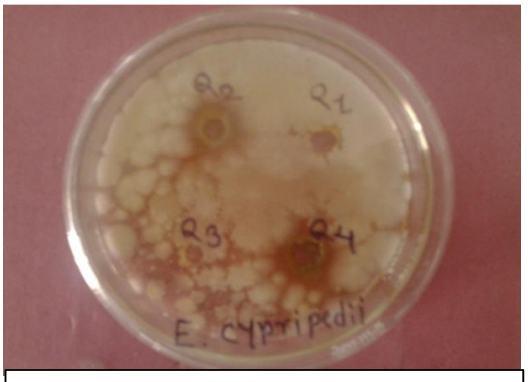


Plate 2 (Ethanolic Extract of (B_1,- *Annona squamosa* B_2- *Punica grantum,* B_3 - *Citrusreticulata,* B_4 *-Manilkara Zapota)*

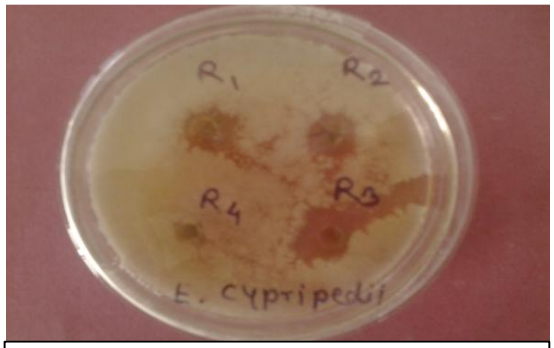


Plate 3(Methanol+chloroform Extract of (C_1,- *Annona* squamosa, C_2 *-Punica grantum,* C_3 *- Citrus reticulata,* C_4 *-Manilkara Zapota)*

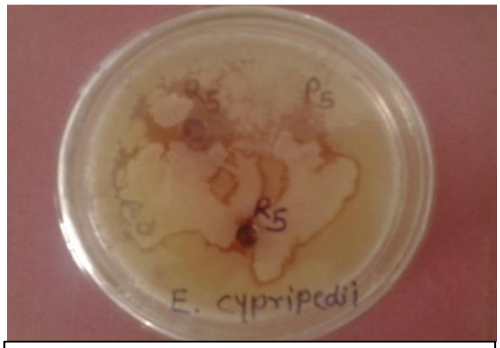


Plate 4(Methanolic Extract of (A_5,- *Psidium gujava,* Ethanolic extract of B_5 - *Psidium gujava, Methnol+chloroform extract of* C_5 - *Psidium gujava)*

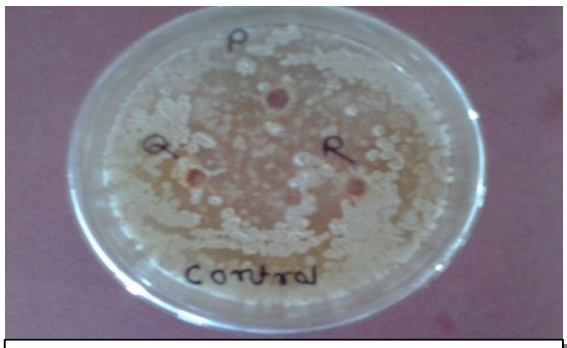


Plate 5 (P- Methanol, Q- 50% ethanol ,R- Methnol+ chloroform)

Fig 8. Zone of inhibition (In mm) of *Erwinia spp.* when screened with different stem extracts prepared in methanol, 50% ethanol methanol + Chloroform against *E.cypripedii.*

http://doi.org/10.29369/ijrbat.2025.010.1.0006