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In-vitro experimental studies of selected biopesticides & their effect on selected plant pathogens

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Abstract

The present work will focus on the importance of plant base biopreparations and their antifungal and antibacterial activity. The plants were selected for preparation of Extracts (biopreparations) to test their broad spectrum of resistance against for the selected plant pathogens as per the literature sources (Anonymous, 1948-76, Dhar., M.L., M.M.Dhar, B.N.Dhawan, B.N.Mehrotra and C.Ray. 1968, Chopra. R. N., Nayar. S. L. and Chopra. I. C. 1986, Bharath Kumar., R.2000.) There are about 15 indigenous plants were selected which are having diversified uses like medicinal, economic, timber and fibre yielding species like , *Ocimum sanctum, Azadarichta indica, Thespesia populnea, Sida cordifolia, Pisonea alba, Nerium indicum, Ficus religiosa, Ricinus communis, Colotropis gigantea, Eucalyptus globulous Achyranthes aspera, Catharanthus roseus, Albizia lebbeck, Typha angustifolia* and *Agave americana.* viz.of Guntur region (Vadlamudi, Tenali etc.). The Antifungal activity was tested against 2 pathogens, which are very much prone to cause severe damage to the commercial crops viz., *Solanum melongena* and *Gossypium herbacium* and the anti bacterial activity was tested against the 2 pathogens, which causes considerable amount of yield loss against the crops like *Lycorpersicum esculentum* and *Citrus limonium.* A total number of 90 plant extracts were prepared as an individual of 45 ethanol, methanol and acetone solvent extracts belongs to the 15 individual plant species (plant parts –leaf, St.b etc.) and another set of 45 plant extracts were prepared in combination ethanol, methanol and acetone solvents. All these plant extracts were subjected against their antibacterial and anti fungal screening analysis, out of these 90 plant extracts (both individual and in combination) 44 plant extracts have been expressed the cognizable zone of expression i.e. 25 mm > 30 mm inhibition. Therefore these combinations were suggested for further analysis of producing a novel broad spectrum of biopesticides for crop protection.

Key-Words: *In-vitro* studies, Biopreparations, Antimicrobial activity, Plant extracts

Introduction

Vignan University (VU) (formerly Vignan's Engineering College is a premier institution affiliated to Jawaharlal Nehru Technological University in Andhra Pradesh). It is having the splendid avenue, imposing buildings and sprawling playgrounds, and the verdure in and around the campus. The college is a virtual haven of rural quiet and idyllic beauty. Since its inception in 1997, VU has been striving to promote high quality standards in technical education & research for the aspirants of Engineering Studies.

Topography

Vignan University is located in the serene environs of Vadlamudi on the Guntur- Tenali highway, about 14 km from Guntur and 11 km from Tenali. The nearest railway station Tenali is located on Chennai – Kolkata trunk line.

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Plant species selected for experimental studies (for Biopreparations), enumeration and description of habit &habitat & chemical composition details of species:

Albizia lebbeck (L.) Benth. (FABACEAE)

Tree. The common name for this is dirasana. The bark yields tannins of condensed type, viz. D-catechin, isomers of leucocyanidin and melacacidin and a new leucoantho-cyanidin, lebbecacidin. It also gives triedelin and t3-sitosterol.Seeds gave crude protein, calcium, phosphorus, iron, niacin, and ascorbic acid, amino acid composition of the protein is: arginine, histidine, leucine & isoleucine lysine, methionine, phenylalanine, threonine, tyrosine, and valine. The flowers contain lupeoL a-and t3-amyrin and a pigment similar to crocetin.

Achyranthes aspera Linn. (AMARANTHACEAE)

Woody Shrubs.Commonly known as Uttareri. It is diuretic, astringent and a blood purifier. It is useful in the diseases, like obesity, piles, phccup, vomiting,

abdominal pain, pruritus and diseases due to ama. (Kaiyadeva Nighantu)

Agave cantala Roxb**.** (AGAVACEAE)

Large Shrubs, The plant, is also known as the American aloe, although it is in a different family from the true aloes.The stems of the plant excrets sap, when they have been chopped with a kinfe it consists, a sweet liquid called *agua miel* ("honey water").

Azadarichta indicia A.Juss. (MELIACEAE)

Neem has a broad spectrum of uses as an anti-fungal agent.The neem tree (*Azadirachta indica*)belongs to *Meliaceae* family, is a fast growing native tree of India. The neem tree is growing in popularity due to its medicinal and fungicidal properties. All the parts of the neem tree are beneficial and the most used parts are the seed kernel, bark and leaves of the tree.Neem oil acts as an effective natural fungicide for plants. The active ingredient found in neem is called *azadireachtin.* Neem extracts act as highly potent natural fungicides for indoor and outdoor plants.

Calotropis gigantea R.Br. (ASCLEPIADACEAE)

Woody Shrubs. *Calotropis gigantea* commonly known as milkweed or swallow-wort, is a common wasteland weed. *Calotropis* is used as a traditional medicinal plant with unique properties. Traditionally *Calotropis* is used alone or with other medicines (Caius 1986) to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhea (Das 1996). The leaves and latex of *C.gigantea* is having excellent fungicidal properties.

Catharanthus roseus Linn. G.Donn. (APOCYNACEAE)

Large Herbs. The plant was popularly known as Madagascar perivinkle.Pharmacological studies have revealed that *C. roseus* contains more than 70 different types of alkaloids and chemotherapeutic agents that are effective in treating various types of considering the medicinal value that this plant has, antibacterial potential in crude extracts of leaves, stem, root and flower against selected clinical bacterial strains.

Eucalyptus globulous Labill (MYRTACEAE)

Trees, Volatile oils from *E. globulous* and its major constituent monoterpene citronellal, possess fungitoxic activities worth exploiting for the biomanagement of plant diseases. *Eucalyptus* volatile oils have potential for the suppression of phytopathogenic fungi. It has weed suppressing and insecticidal properties.

Ficus religiosa L. (MORACEAE)

Tree. Commonly known as Sacred Fig. *Ficus religiosa L.* They are large deciduous trees, Various parts of the plant like bark, leaves, tender shoots, fruits, seeds, and latex are medicinally important. The bark contains tannin, rubber and wax. The plant parts are used in diseases of blood, vagina, uterus and leukorrhea, burning sensation, gonorrhea, diarrhea, dysentery, hemorrhoids, gastrohelcosis. The bark is used in inflammations, swelling of neck, gonorrhea, scabies, mouth wash fortoothache and for strengthening gums, and steeped freshly burnt bark has been said to cure cases of obstinate hiccup. The latex is used in inflammations and hemorrhages.

Nerium indicum L. (APOCYNACEAE)

Large Shrubs. Large shrubs. The bark contains scopoletin, scopolin, tannins, red coloring matter, a aromatic oil, wax and flobefin and a yellow colored stable oil. The roots contain bitter glycosides, fenolinic acid and a aromatic oil, glycosided, neriodorin, neriodorein, and karabin. It contains neriodin, nerium D, rutin and anhydro-oleandrin. Roots are astringent, anthelmintic, aphrodisiac, stomachic, febrifuge.Also have diuretic, emetic, expectorant, cardiotonic and anticancer.Useful in cardiac asthma, joint pains, leprosy and ulcers.Leaves are powerful repellent and in treatment of scabies and to reduce swellings

Ocimum sanctum Linn. (LAMIACEAE)

Moderate Herb, commonly refered as Tulsi. It belongs to *Lamiaceae* family Considered as main source of potential active metabolites essential oil of *Ocimum* has antifungal activity. It has non cyclic sesquiterpens, phenols, eugenol, alpha-pinen and terpinene chemicals which inhibit the growth of fungal pathogens.

Pisonia alba Span. (NYCTAGINACEAE)

Large Shrubs (moderate sized trees). Commonly known as lettuce tree and evergreen foliage tree. The leaves are also carminative (expels flatulence). Leaves coated with eau de cologne are used to rub on elephantoid swellings (anti-inflammatory). The plant consists the popular chemical constituents like consists, secopisonic acid, pisoninol pisoninol II, pisoquinoline and pisodienone etc.

Ricinus communis L. (EUPHORBIACEAE)

Moderate Trees. The castor oil plant, Methanolic extracts of the leaves of *Ricinus communis* were use Antimicrobial testing against pathogenic bacteria. Antihistamine and anti-inflammatory properties found in ethanolic extract of Ricinus communis root bark. Extract of Ricinus communis, exhibited acaricidal and insecticidal activities.

Sida cordifolia L. (MALVACEAE)

Woody Shrubs. The common name for *S.cordifolia* is Balavatya. It consists ephedrine and pseudo-ephedrine along with other compounds as active ingredients. Stem of *Sida cordifolia* contains a number of active compounds, including small amounts of an essential oil, 1-2% alkaloids composed mainly of ephedrine and

pseudoephedrine.These active ingredients acts as pesticides.

Thespesia populnea L. (MALVACEAE)

Tree, Milo is a tree of coastal regions. It is commonly known as Gangaravi/Gangareni chettu.*Thespesia populnea* widely used by the traditional practitioners for the treatment of infectious diseases. The plant extracts have been shown to have anti-bacterial and anti-viral activity. It possess fungitoxic activities worth exploiting for the biomanagement of plant diseases.

Typha angustifolia L. (TYPHACEAE)

Typha (meaning "marsh" in Greek) Aquatic tuff shrubs. Plant contains three steroids [b-sitosterol, (20S) 24-methylenlophenol and stigmast-4-ene-3, 6-dione] and three fatty acids [a– linolenic, linoleic, and an unidentified $C_{8\ 2}$]. Roots are rich in polysaccharides. Flavonoids are present in shoots and flowering heads. Xu *et al. (*1986) isolated seven crystalline compounds from the inflorescence of *Typha angustifolia*. These compounds were vanillic acid, E-p-hydroxy-cinnamic acid, protocatechuic acid, E-Pro-penoic acid-3-(hydroxyphenyl)-2,3-dihydropropyl ester, succinic acid, p-hydroxybenzaldehyde and D-mannitol.Medicinally active principles in *T. angustifolia* have been mainly identified as flavonoids (Gao *et al.*, 1998; Xi and Li, 2000).

Material and Methods

Vignan University has campus with a good number of plants. It includes landscaping gardens, exotic elements and natural forest elements, includes rare and endemic categories of trees, shrubs, herbaceous members, climbers and a good number medicinal plants like *Ocimum sanctum, Azadarichta indica, Thespesia populnea, Sida cordifolia, Pisonea alba, Nerium indicum, Ficus religiosa*, etc. An inventory experimental studies were conducted on selected most promising plant species which are having utilization of domestic, commercial importance of plant based biopreparations. Methodology was adopted for the above mentioned studies are as per standard literature sources.

The present work was conducted in School of Biotechnology, Microbiology lab Vignan University, Vadlamudi to determine the antifungal and antibacterial activity of *Ocimum sanctum, Azadarichta indica, Thespesia populnea, Sida cordifolia, Pisonea alba, Nerium indicum, Ficus religiosa, Ricinus communis, Calotropis gigantea, Eucalyptus globulous Achyranthes aspera, Catharanthus roseus, Albizia lebbeck, Typha angustifolia* and *Agave americana.* against two selected fungal pathogens viz.,*Colletotrichum .melongena* and *Cercospora.gossypina* & against two selected bacterial pathogens viz.,*Pseudomonas syringae* and *Xanthomonas citri* in ethanol methanol and acetone by employing food poisoning technique (Naz.*et al*., 2006).

Extraction of Disease Causing Organism/s & Preparation of Test Plates:

Pathogens were isolated from infected Brinjal,Cotton,Tomato and Citrus leaves with visible symptoms of round spot with brown circles surrounded by red and yellow halos the central portion will be white for Cotton, on tomato leaves, symptoms appear as black specks and Plants infected with citrus canker have characteristic lesions on leaves, stems, and fruit with raised, brown, water-soaked margins, usually with a yellow halo or ring effect around the lesion. Diseased leaf samples were surface sterilized with 5% Chlorox for, one minute and washed three times with sterilized distilled water.

Preparation of pure culture, Identification and confirmation of isolated organisms:

The affected portion of the leaf is carefully separated using sterile knife and crushed separately using mortor and pestle by slowly adding sterile distilled water and then the extract is filtered using sterile Whattmann No.1 filter paper, the extract is verified for the presence of organism under microscope after conformation the extracts are carried for further experiments which are stored at 4 ^{0}c. The identification and confirmation of isolated microorganisms (both bacterial and fungal species) has been done by the standard procedures suggested in the literature sources (Prescott and Dunn(2004), Aneja (2007).

Preparation of Plant Extracts (Biopreparations):

Collection and preservation of plants samples and preparation of extracts:

Fresh leaves of *Ocimum sanctum(*Oc.s.), *Azadarichta indica(*Az.i),*Thespesia populnea(*Ths.p.) , *Sida cordifolia*(Sd.c),*Pisonea alba*(Pa.), *Nerium indicum*(N.i.), *Ficus religiosa*(Fi.r), *Ricinus communis* (R.c.),*Calotropis gigantea*(C.g.), *Eucalyptus globulous*(Eu.g.), *Achyranthes aspera*(Ach.a), *Catharanthus roseus*(Cath.r.), *Albizia lebbeck*(Al.le.),*Typha angustifolia*(Ty.an.) and *Agave Americana*(Ag.a) collected viz.of Guntur region (Vadlamudi, Tenali etc.)

These were washed with tap water and cut into small pieces which are air dried for 2-3 days at room temperature to eliminate surface moisture. Dried leaves were grinded separately in an eclectic grinder to obtain powder which was then kept in plastic bags for further use.

Five gram of the dried powdered plant were soaked separately in 50ml of ethanol, methanol and acetone. These extracts were boiled on water bath at 70°c for 24-48 hours .The ethanolic, methanolic and acetone extracts were squeezed and filtered using filter paper and stored at room temperature for further use.

Preparation of Media and Screening of Antimicrobial activity:

a) Media & Microorganisms:

The suitable culture media was prepared by dissolving the below mentioned ingredients for the respective microorganisms. The contents were autoclaved at 15lbs for 15 min. microorganisms are taken as.,*Pseudomonas syringae* and *Xanthomonas citri* (bacterial species) and .,*Colletotrichum .melongena* and *Cercospora gossypina* (fungal species)For antimicrobial activities of plant extracts.

b) Preparation of Sterile Paper Disks:

Using an ordinary office two-hole puncher, paper disks with approximate diameter of 6.3 mm. were punched out one by one from a sheet of blotting paper, the disks were placed in boiling tubes then autoclaved for 15 minutes at 15 lbs. pressure and allowed to cool.

Medium for Bacterial Species: Nutrient Broth/Nutrient Agar Medium (NBM/NAM) composition:

Peptone -5gr
Beef extract -3gr
Agar -5 gr
Distilled water-1000 ml
P^H -7

Medium for Fungal Species:

Potato Dextrose Agar Medium (PDAM) ingredients:

Potato -20 gr
Dextrose -20 gr
Agar -20 gr
Streptomysin -30 gr
Distilled water -1000 ml
P^H -7

Preparation of Test Plates for Antimicrobial Screening Tests:

The Nutrient Agar (NA) and Potato Dextrose Agar (PDA) test plates (Petridishes) were prepared by pouring about 15 ml of the medium. These test plates were placed under aseptic conditions at 4^0 C for 24 hours to control sterility. After solidifying the media (NA &PDA). The inoculums (bacteria 24 hrs and fungi 48 hrs.) Stock cultures were uniformly spread on their respective test plates. The filter paper discs were prepared in ethanol, methanol (M) and acetone (A) extracts as taken for control.

The filter paper discs are carefully placed on the prepared culture test plates and incubated them at appropriate temperature for bacteria at 37^0 C for 24 hrs. And fungi 30^0 C for 48 hrs. After the incubation period. The test plates are examined for inhibitory zones are recorded. All determinants were made atleast in triplicate for each of the test organisms in different extracts are also recorded.

Results and Discussion

A total no. of 90 ethanol, methanol and acetone solvent extracts belongs to the 15 plant species of both individual and with combinations (Lf.) were subjected for antifungal and antimicrobial screening, in that all the 90 samples are exhibited positive inhibition zone activity . The observations are recorded and they have been categorized into high or maximum zone (cognizable inhibitory zone) (i.e.25-30 mm inhibition zone) in 43 samples of (Ethanol/Methanol/Acetone extracts), moderate inhibition zone of expression in 84 samples of (E/M/A extracts) (i.e. 15-20 mm inhibition zone) and minimal inhibition zone of expression in 176 samples of (i.e. < 15 mm inhibition zone).

The inhibitory activity i.e.25to 30 mm zone of expression for plant samples (Ethanol extracts) for individual in 18 samples, in combination 3samples i.e. in total= 21samples. 25 to 30mm zone of expression for plant samples (Methanol extracts) for individual in 9 samples, in combination 2 samples i.e. in total=11 samples and 25 to 30mm zone of expression for plant samples (Acetone extracts)for individual in 8 samples and combination 2samples i.e. in total =10 samples. [**Annexure (**Tables:**1,2,3,4,5,6,7& 8)]**

Ethanol extracts are comparatively effective more than those of methanol extracts. The methanol extracts similarly than those of acetone extracts are shown high inhibitory activity in Gram +ve bacteria and comparatively less in both Gram –ve bacteria.

Conclusion

The present work will focus on the importance of plant base biopreparations and their antifungal and antibacterial activity. The plants were selected for preparation of Extracts (biopreparations) to test their broad spectrum of resistance against for the selected plant pathogens as per the literature sources.

The Antifungal activity was tested against 2 pathogens, which are very much prone to cause severe damage to the commercial crops viz., *Solanum melongena* and *Gossypium herbacium* and the anti bacterial activity was tested against the 2 pathogens, which causes considerable amount of yield loss against the crops like *Lycorpersicum esculentum* and *Citrus limonium.* A total number of 90 plant extracts were prepared as an individual of 45 ethanol, methanol and acetone solvent

extracts belongs to the 15 individual plant species (plant parts –leaf, St.b etc.) and another set of 45 plant extracts were prepared in combination ethanol, methanol and acetone solvents.All these plant extracts were subjected against their antibacterial and anti fungal screening analysis, out of these 90 plant extracts (both individual and in combination) 44 plant extracts have been expressed the cognizable zone of expression i.e. 25 mm > 30 mm inhibition[**Annexure (**Tables:**1,2,3,4,5,6,7& 8)].**Therefore these combinations were suggested for further analysis of producing a novel broad spectrum of biopesticides for crop protection.

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Table 1: Showing the antimicrobial inhibition activity (zone of inhibition 10mm to >/= 25mm)-plant extracts are subjected to *C.melongena*

Organism	Plant extract	10 to 12mm			12 to15 mm			15 to 20mm			20 to25mm			>25 mm (25-30mm)		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
C. melongena	Ocs	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–
C. melongena	Azi	–	–	–	–	–	–	–	–	–	–	-	–	–	–	–
C. melongena	Thsp	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–
C. melongena	Sdc	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–
C. melongena	Pa	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. melongena	Ni	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–
C. melongena	Fi.r	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. melongena	R.c	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–
C. melongena	C.g	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+
C. melongena	Eug	–	–	–	–	–	+	–	+	–	+	–	–	–	–	–
C. melongena	Ach.a	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–
C. melongena	Cath.r	–	–	–	–	–	+	–	–	–	+	+	–	–	–	–
C. melongena	Al.le	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C.	Ty.an	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–

melongena																
C. melongena	Ag.a	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–

Table 2: Showing the antimicrobial inhibition activity (zone of inhibition 10mm to >/= 25mm)-plant extracts are subjected to *C.gossipina*

Organism	Plant extract	10 to 12mm			12 to15 mm			15 to 20mm			20 to25mm			>25 mm (25-30mm)		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
C. gossipina	Ocs	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	Azi	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	Thsp	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	Sdc	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
C. gossipina	Pa	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
C. gossipina	Ni	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
C. gossipina	Fi.r	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	R.c	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–
C. gossipina	C.g	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	Eug	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	Ach.a	–	–	–	–	–	–	+	+	–	+	–	–	–	–	–
C. gossipina	Cath.r	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–
C. gossipina	Al.le	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	Ty.an	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+
C. gossipina	Ag.a	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–

Table 3: Showing the antimicrobial inhibition activity (zone of inhibition 10mm to >/=25mm)-plant extracts are subjected to Pseudomonas *syringae*

Organism	Plant extract	10 to 12mm			12 to15 mm			15 to 20mm			20 to25mm			>25 mm (25-30mm)		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
P. syringae	Ocs	–	–	–	–	–	–	–	–	–	+	–	–	+	+	–
P. syringae	Azi	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
P. syringae	Thsp	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
P. syringae	Sdc	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+
P. syringae	Pa	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
P. syringae	Ni	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+

P. syringae	Fi.r	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
P. syringae	R.c	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
P. syringae	C.g	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
P. syringae	Eug	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+
P. syringae	Ach.a	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+
P. syringae	Cath.r	–	–	–	–	–	+	–	–	–	–	+	–	+	–	–
P. syringae	Al.le	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
P. syringae	Ty.an	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
P. syringae	Ag.a	–	–	–	–	–	–	–	+	–	–	+	+	–	–	–

Table 4: Showing the antimicrobial inhibition activity (zone of inhibition 10mm to >/= 25mm)-plant extracts are subjected to *Xanthomonas citri*

Organism	Plant extract	10 to 12mm			12 to15 mm			15 to 20mm			20 to25mm			>25 mm (25-30mm)		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
X. citri	Ocs	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Azi	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Thsp	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Sdc	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Pa	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Ni	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
X. citri	Fi.r	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X citri	R.c	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–
X. citri	C.g	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Eug	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Ach.a	–	–	–	–	–	–	+	+	–	–	–	+	–	–	–
X. citri	Cath.r	–	–	–	–	–	–	–	–	–	–	+	–	+	–	+
X. citri	Al.le	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Ty.an	–	–	–	–	–	–	–	–	–	–	+	–	+	–	+
X. citri	Ag.a	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–

Table 5: Showing the antimicrobial inhibition activity (zone of inhibition 10mm to >/= 25mm)-plant extracts are subjected to *C.melongena*

Organism	Plant extract	10 to 12mm			12 to15 mm			15 to 20mm			20 to25mm			>25 mm (25-30mm)		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
C. melongena	R.c+Ca.g	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–
C.	Thsp+	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–

melongena	P.a															
C. melongena	Ca.g+A.le	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
C. melongena	Ca.g+Eu.g	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–
C. melongena	Az.i+Ca.g+P.a	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–
C. melongena	Ca.g+Oc+S.c	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
C. melongena	R.c+Eu.g	+	–	–	–	–	+	–	+	–	–	–	–	–	–	–
C. melongena	R.c+S.c	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–
C. melongena	R.c+Thsp	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–
C. melongena	Ty+R.c	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–
C. melongena	Ty+A.as	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–
C. melongena	Ty+Thsp	+	–	–	–	–	+	–	+	–	–	–	–	–	–	–
C. melongena	A.a+Ty	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–
C. melongena	Ag.a+C.r	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
C. melongena	Ag.a+N.i	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–

Table 6: Showing the antimicrobial inhibition activity (zone of inhibition 10mm to >/= 25mm)-plant extracts are subjected to *C.gossipina*

Organism	Plant extract	10 to 12mm			12 to15 mm			15 to 20mm			20 to25mm			>25 mm (25-30mm)		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
C. gossipina	R.c+Ca.g	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	Thsp+P.a	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–
C. gossipina	Ca.g+A.le	–	–	+	+	–	–	–	+	–	–	–	–	–	–	–
C. gossipina	Ca.g+Eu.g	–	–	+	–	+	–	–	–	–	–	–	–	+	–	–
C. gossipina	Az.i+Ca.g+P.a	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
C. gossipina	Ca.g+Oc+S.c	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–
C. gossipina	R.c+Eu.g	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–
C. gossipina	R.c+S.c	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–
C. gossipina	R.c+Thsp	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
C. gossipina	Ty+R.c	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–
C. gossipina	Ty+A.as	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–
C. gossipina	Ty+Thsp	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–
C. gossipina	A.a+Ty	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–
C. gossipina	Ag.a+C.r	–	+	–	–	–	–	–	–	+	+	–	–	–	–	–
C. gossipina	Ag.a+N.i	–	–	–	+	–	–	–	+	–	–	–	–	–	–	+

Table 7: Showing the antimicrobial inhibition activity (zone of inhibition 10mm to >/= 25mm)-plant extracts are subjected to *Pseudomonas syringae*

Organism	Plant extract	10 to 12mm			12 to15 mm			15 to 20mm			20 to25mm			>25 mm (25-30mm)		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
P. syringae	R.c+Ca.g	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
P. syringae	Thsp+P.a	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
P.	Ca.g+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–

syringae	A.le															
P. syringae	Ca.g+ Eu.g	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–
P. syringae	Az.i+C a.g+P.a	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
P. syringae	Ca.g+ Oc+S.c	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
P. syringae	R.c+E u.g	+	–	–	–	–	+	–	+	–	–	–	–	–	–	–
P. syringae	R.c+S. c	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–
P. syringae	R.c+T hsp	–	–	–	+	+	+	–	+	–	–	–	–	–	–	–
P. syringae	Ty+R. c	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–
P. syringae	Ty+A. as	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–
P. syringae	Ty+Th sp	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
P. syringae	A.a+T y	+	+	–	+	–	+	–	–	–	–	–	–	–	–	–
P. syringae	Ag.a+ C.r	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–
P. syringae	Ag.a+ N.i	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–

Table 8: Showing the antimicrobial inhibition activity (zone of inhibition 10mm to >/= 25mm)-plant extracts are subjected to *Xanthomonas citri*

Organism	Plant extract	10 to 12mm			12 to15 mm			15 to 20mm			20 to25mm			>25 mm (25-30mm)		
		E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
X. citri	R.c+C a.g	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Thsp+ P.a	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Ca.g+ A.le	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	Ca.g+ Eu.g	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–
X. citri	Az.i+C a.g+P.a	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–
X. citri	Ca.g+ Oc+S.c	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
X. citri	R.c+E	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–

	u.g															
X citri	R.c+S.c	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–
X. citri	R.c+Thsp	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–
X. citri	Ty+R.c	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–
X. citri	Ty+A.as	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–
X. citri	Ty+Thsp	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
X. citri	A.a+Ty	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–
X. citri	Ag.a+C.r	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–
X. citri	Ag.a+N.i	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–



Albizia lebbeck (L.) Benth. ***Achyranthes aspera*** Linn. ***Agave cantala*** Roxb



Azadarichta indicia A.Juss. ***Calotropis gigantea*** R.Br. ***Catharanthus roseus*** Linn. G.Donn.

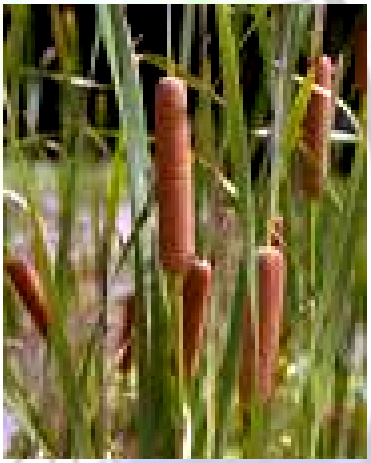


Eucalyptus globulous Labill. ***Ficus religiosa*** L. ***Nerium indicum*** L.



Ocimum sanctum L. ***Pisonia alba*** Span. ***Ricinus communis*** L.





Sida cordifolia L. ***Thespesia populnea*** L. ***Typha angustifolia*** L.