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Green synthesis of copper and zinc nanoparticles from *Chromolaena odorata* and *Justicia adhatoda* and evaluation of their antifungal activity against *Fusarium oxysporum cubense*: An overview

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Abstract

Soil borne diseases which are caused to various plants include a wide variety of soil microbes like fungi and bacteria, among which Fusarium wilt is one such disease caused by *Fusarium oxysporum cubense* in banana plants. Wilt disease or the panama disease of plant is among the most destructive disease of banana in the tropics and even the control methods like field sanitation, soil treatments and crop rotations have not been a long term control for this disease. An alternative method of treating *Fusarium oxysporum* was adopted by using various natural plant leaves of *Chromolaena odorata* and *Justicia adhatoda*. Nanoparticles are small particles with a dimension of 10^{-9} and 10^{-10} . Green synthesis is a new method developed for the synthesis of nanoparticles which is small in size, large surface area and eco-friendly. Leaf extracts of these plants were used for synthesis of copper and zinc nanoparticles, as nanoparticles are powerful antimicrobial agents. The extract is prepared with a stock solution of 100mM copper sulphate and 100mM zinc sulphate. The leaf extracts were prepared with 5 solvents (Distilled water, Propane, Hexane, Acetone and Methanol). The action of plant leaves were observed by the zone of inhibition obtained with a concentration of 50, 100 and 150 μ l respectively. The result was more in copper nanoparticles of leaf extract as compared to the zinc nanoparticles of particular leaf extracts but the zinc particles with methanol and propane showed good result with particular leaves. In dried condition of leaves copper nanoparticles with propane as solvent exhibited a greater zone of inhibition. Moreover the solvent, methanol showed good results with both zinc and copper nanoparticles. The synthesized nanoparticle were characterized by UV-VIS spectrophotometry to confirm the formation of nanoparticles. Green synthesis is used namely because of low cost, simple, use of less toxic materials, most important is eco-friendly.

Keywords: Nanoparticles, *Fusarium oxysporum cubense*, Panama wilt, *Chromolaena odorata*, *Justicia adhatoda*; Antifungal assay, PDA

1. Introduction

Nanotechnology is an emerging field of science. It has increased applications in diverse area for the development of new materials at nanoscale levels (Paul *et al.*, 2015) [61]. Nanotechnology mainly consists of the processing of, separation, consolidation, and deformation of materials by one atom or one molecule (Prasad *et al.*, 2008) [67]. Nanoparticles has 1-100 nm in size and they possess novel physical and chemical properties (Sajeshkumar *et al.*, 2015a; Sajeshkumar *et al.*, 2015b) [74-75]. Nanoparticles bear antibacterial properties (Hajipour *et al.*, 2012) [26].

Nanoparticles play important role in fighting against disease causing microbes. Nanoparticles are very minute particles. Due to large surface volume ratio; renewable surface and varying micro electrode potential values nanoparticles are largely used as catalysts also (Din and Rehan, 2017) [11]. There are different types of nanoparticles including; silver, copper, zinc (metal nanoparticles). Nowadays humans face dangers infections due to pathogenic microbes. Nanoparticles can overcome this problems. Nanoparticles have antibacterial property. Metal nanoparticles such as silver, copper and zinc has inhibitory effect on microorganisms.

Nanotechnology is an emerging field which makes an impact on human life such as health, food, chemical and energy industries, environmental and space industries etc.

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Various methods to synthesize nanoparticles include sol gel method, chemical reaction, solid state reaction and co precipitation. Another method used is the green synthesis method which is one of the most appropriate method used in recent years. This method have several advantages namely low cost, simple, use of less toxic materials, most important is eco-friendly. The metal nanoparticles such as Ag, Cu etc., are found to have antibacterial and antifungal activity. This effect of metal nanoparticles has been attributed to their small size, and high surface to volume ratio, which allow them to interact closely with microbial membranes and it is not merely due to the release of metal ions in solutions. The antibacterial and antifungal properties of the metal nanoparticles find applications in various fields such as medical instrument, and devices, water treatment and food processing. Some of the methods to prepare nanoparticles is by using the methods such as thermal reduction, vacuum vapor deposition, microwave irradiation methods, chemical reduction, and laser ablation. All these methods use oxygen-free atmosphere to synthesize copper, zinc or aluminium nanoparticles because it easily oxidizes. Nanoparticles have various applications in optoelectronics, nanodevices, nanoelectronics, nanosensors, information storage etc. Among various metal particles, copper nanoparticles have attracted more attention because of their catalytic, optical, electrical and antifungal/antibacterial applications (Ramyadevi *et al.*, 2012) [73].

Soil borne diseases which are caused to various plants include a wide variety of soil microbes like fungi and bacteria, among which Fusarium wilt is one such disease caused by *Fusarium oxysporum cubense* in banana plants. A decrease in the pathogen growth in soil is manipulated through agro-ecosystem, which focuses on the depletion of various soil borne diseases in banana plants (Shen *et al.*, 2019) [78]. Bananas are an important source of living for farmers across wet tropics and subtropics, including various countries like Americas, Africa, Southeast Asia and the Pacific. Although it is a commercial crop in the world, but it is considered that 87% of the banana production is for local food consumption (Langhe, *et al.*, 2009) [9].

Wilt disease or the panama disease of plant is among the most destructive disease of banana in the tropics and even the control methods like field sanitation, soil treatments, crop rotations and organic amendments have not been a long term control for this disease. Many potential biocontrol agents can be developed against *Fusarium oxysporum cubense* by understanding the interactions between the biocontrol and fungal pathogen (Getha and Vikineswary, 2002) [20]. Bananas are rich sources of both simple and complex carbohydrates, and of the vitamins ascorbic acid, B6, carotene, niacin, riboflavin, and thiamin. They are also excellent sources of potassium. Moreover, bananas are easily digestible, offering access to food energy faster than apples and meats.

The reddish brown discoloration of the xylem, develops in feeder roots, the initial sites of infection shows the first internal symptom. Vascular discoloration progresses to the rhizome, is most prominent where the stele joins the cortex. The younger leaves wilt and collapse until the entire canopy consists of dead or dying leaves. *F. oxysporum* cannot be morphologically distinguished easily. There colonies grow 4-7 mm on potato dextrose agar at 25 °C with abundant white to purple mycelium. (Ploetz, 2005) [65] Both pathogenic and nonpathogenic strains of *F. oxysporum* are found in agricultural soils throughout the world, and it is these populations that have received the most attention from researchers. It is not a pathogen of plants in native situations

and the grasslands often support large populations of *F. oxysporum*, yet grasses, whether cultivated or native, are not known to be hosts to pathogenic strains of this fungus. Moreover understanding the evolution of pathogens in *F. oxysporum* species will ultimately require a detailed characterization of the relationships between diverse pathogenic and nonpathogenic forms in this species (Gordon and Martyn, 1997) [22].

1.1 Green synthesis of nanoparticles

Synthesis of nanoparticles using biological methods is referred as greener synthesis of nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, and safe for human therapeutic use (Kumar *et al.*, 2009) [40]. Metals like silver, copper and zinc has inhibitory effect on microbes. Biological synthesis of metallic nanoparticles is inexpensive single step and eco-friendly methods. The plants and seeds are used successfully in the synthesis of various greener nanoparticles such as copper, silver, and zinc oxide (Kuppusamy *et al.*, 2014; Mishra *et al.*, 2014) [43, 50].

1.2 Application of nanoparticles

Nanoparticles has various applications. Nanoparticles have been used for constructing electrochemical and biosensors (Luo *et al.*, 2006) [47]. Metal nanoparticles embedded paints have good antibacterial activity (Kumar *et al.*, 2008) [41]. Current research is going on regarding the use of magnetic nanoparticles in the detoxification of military personnel in case of biochemical warfare (Salata, 2004) [76].

One of the major opportunities for nanoparticles in the area of computers and electronics is their use in a special polishing process, chemical-mechanical polishing or chemical mechanical planarization, which is critical to semiconductor chip fabrication (Elechiguerra *et al.*, 2005) [15].

Magnetic nanoparticles are also used in targeted therapy where a cytotoxic drug is attached to a biocompatible nanoparticle for tumour cell treatment (Pankhurst *et al.*, 2003). Porous nanoparticles have been used in cancer therapy. Bioremediation of radioactive wastes from nuclear power plants and nuclear weapon production such as uranium has been achieved using nanoparticles (Duran *et al.*, 2007) [13].

1.3 Copper nanoparticles

Copper nanoparticles have high optical, catalytic, mechanic and electrical properties. They are cheap high yielding and have short reaction time under normal reaction condition. Copper nanoparticles have anti-microbial activities against various bacterial and fungal strain from any researchers (Patravale *et al.*, 2004) [60]. It is used in various fields including agricultural, industrial, engineering and technical fields. Effective anti-bacterial activities are exhibited by copper nanoparticles. They are cost effective and have efficient bio synthesize techniques. Copper nanoparticles have less cost than silver and gold nanoparticles.

1.4 Zinc nanoparticles

Zinc nanoparticles have wide application; various synthetic methods have been employed to produce ZnNPs (Chen *et al.*, 2007) [6]. Zinc nanoparticles can produced from zinc oxide and zinc sulphate. Zinc nanoparticles has several medicinal uses, which harm skin, stomach, intestine and lymphatic system and they probably induces tumours. Zinc nanoparticles has antibacterial effect on microbes, and it mainly depends up on the size and the presence of visible light. Zinc

nanoparticles are used in the optical devices, sensors, catalysis, biotechnology, DNA labelling, drug delivery, medical, chemical and biological sensors (Devasenan *et al.*, 2016)^[10].

1.5 Antimicrobial activity

Anti-microbial agent is a substance that kills microorganisms or stops their growth. Anti-microbial medicines are grouped according to the micro-organisms they act. Antibiotic are used against bacteria, antifungal are used against fungi. They are also classified on the basis of their function. The agents that kill microbes are called microbicidal; those that merely inhibit their growth are called biostatic (Al Juhaiman *et al.*, 2010)^[1]. The use of anti-microbial agents for the treatment of infection is known as anti-microbial therapy. The use of antimicrobial medicines for the prevention of infection is known as antimicrobial prophylaxis.

1.6 Antifungal activity

Antifungals are used to treat fungal infections. The drug toxicity to humans and other animals from antifungals is generally high. They comprise a large and diverse group of drugs used to treat fungal infections. The mechanism of action of the antifungals include inhibition of fungal membrane and cell wall synthesis, alterations of fungal membranes, effects of microtubules and inhibition of nucleic acid synthesis. Antifungal activities potentially offer solution to the problem of antibiotic resistance. The antifungal medication is also called as antimycotic medication, a pharmaceutical fungicide used to treat and prevent mycosis and serious systematic infections. They are made to acts against plants, animals and humans. The modern era of antifungal therapy by the introduction of oral griseofulvin and topical chlormidazole in 1958 and the subsequent introduction of IV AmB in 1960. Antifungal creams, liquids and sprays are used to treat fungal infections.

1.7 Agar well diffusion

Agar well diffusion test is used for antifungal assay. The well that cut on the solidified agar act as pour for loading sample. The agar that is inoculated with test organism after overnight incubation may show zone of inhibition. The sample that is diffused in the agar inhibits the growth of microbes.

1.8 Objectives

Synthesis of copper and zinc nanoparticles using leaf extracts of two different plants; Communist palla (*Chromolaena odorata*), Adalodakam (*Justicia adhatoda*) and determine the antifungal properties of these nanoparticles against *Fusarium oxysporum cubense*.

1.9 Scope of the study

The study would enlighten the medical and pharmaceutical applications various green synthesised nanoparticles applications against *Fusarium oxysporum cubense* which could be further explored.

2. Review of literature

Copper nanoparticles widely used due to their superior, optical, electrical, antifungal/antibacterial and biomedical applications. Copper nanoparticles have superior antibacterial activity as compared to silver nanoparticles. Because copper is highly toxic to microorganisms (Singh, 2017).

The antimicrobial activity mainly tested for drug discovery

and prediction of therapeutic outcome. Agar disc diffusion and agar well diffusion are two methods used to evaluate antimicrobial activity (Balouiri *et al.*, 2016)^[2].

2.1 *Chromolaena odorata* (Communist palla)

Chromolaena odorata is a diffuse, scram-bling shrub that is mainly a weed of plantation crops and found in southern Asia and western Africa. It forms a bush 3–7 m in height when growing in the open. It has soft stems with the base being woody. Extracts from its leaves have shown to be beneficial for treatment of wounds. The plant is hairy and glandular and the leaves have a pungent, aromatic odour when crushed. Traditionally, the fresh leaves have been used for many years in tropical countries for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis. The extracts also showed inhibitory actions against various microbes. It is also used to enhance hemostasis and blood coagulation. The extract from *C. odorata* was scientifically demonstrated to have a strong anti-oxidant effect. (Phan, Wang *et al.*, 2001)^[64]

2.1.1 Taxonomical classification (Communist palla)

Kingdom: Plantae-- planta, plantes, plants, vegetal
Subkingdom: Viridiplantae
Superdivision: Embryophyta
Division: Tracheophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Chromolaena
Species: *Chromolaena odorata*

2.2 *Justicia adhatoda* (Adalodakam)

Justicia adhatoda is also known as Malabar nut or Vasaka and is a 1–6 m tall, evergreen, perennial shrub. It is distributed in the sparse tree canopy habitats in tropical to sub-tropical areas. The plant has played an important role both in the ancient Indian system of medicine – the Ayurveda and also in modern pharmaceutical and cosmetics industries. Over time, the habitat of Malabar nut has shrunk due to an ever-increasing demand on land for cultivation, industry, human habitation, and other economic development activities. It is a medicinal plant widely used in Siddha Medicine, Ayurvedic, homeopathy and Unani systems of medicine. Used to cure cold,cough, asthma,, joint pain, lumber pain,sprains, cough, eczema, malaria, rheumatism, swellings bronchitis and tuberculosis and the leaf juice is shown to cure diabetes,diarrhea, dysentery and liver relevant disorders. (Yang, Kushwaha *et al.*, 2013)^[84] The plant has various antioxidant and antibacterial properties which makes them one of the herbal medicinal plant.

2.2.1 Taxonomical classification (Malabar nut; Adalodakam)

Kingdom: Plantae-- planta, plantes, plants, vegetal
Subkingdom: Viridiplantae
Superdivision: Embryophyta
Division: Tracheophyta
Class: Magnoliopsida
Subclass: Magnoliidae
Order: Lamiales
Family: Acanthaceae
Genus: Justicia
Species: *Justicia adhatoda*

3. Hypothesis

The current research work is based on the following hypothesis

- 1) Plant extracts of Communist palla (*Chromolaena odorata*), Adalodakam (*Justicia adhatoda*), could be used as antifungal agents.
- 2) These plant extracts could be used in formulating nanoparticles (copper and zinc) and their antifungal activity vary widely.

4. Materials and Methods

4.1 Study area

Kerala state covers an area of 38,863 km² with a population density of 859 per km² and spread across 14 districts. The climate is characterized by tropical wet and dry with average annual rainfall amounts to 2,817 ± 406 mm and mean annual temperature is 26.8°C (averages from 1871-2005; Krishnakumar *et al.*, 2009)^[39]. Maximum rainfall occurs from June to September mainly due to South West Monsoon and temperatures are highest in May and November.

4.2 Sample collection

Samples of Communist palla (*Chromolaena odorata*), Adalodakam (*Justicia adhatoda*), were collected from Ramapuram, Kottayam district of Kerala State, India. The leaves were thoroughly cleaned using double distilled water. The samples were dried in hot air oven at 60°C for 48 hrs and later stored in air tight polyethylene zipper bag for analysis.

4.3 Extraction method

4.3.1 Dried extraction

About 1 g of dried samples are taken in a test tube to which 9ml of distilled water, propane, hexane, acetone or methanol is added. The mixture is mixed well and is kept for half an hour. It is then filtered using a filter paper into a container which is then stored at 4°C for further use. The obtained dried leaf extract shows different colour in different solvents.

4.3.2 Fresh extraction

Leaf extract is prepared with 10 g of fresh leafs (*Chromolaena odorata*, *Justicia adhatoda*) thoroughly washed with tap water and then with DH₂O and cut into small pieces. It is then crushed in a pistil and motor by adding 30 ml of DH₂O. It is then filtered using a filter paper into a container and is then stored at 4°C for further use.

4.4 Synthesis of nanoparticles

4.4.1 Copper nanoparticles

The stock solution is prepared by dissolving 2.49 g of Copper sulphate (CUSO₄) in 100 ml of DH₂O. Add 9ml of the 100mM CUSO₄ solution to 1ml of the leaf extract and is allowed to react in room temperature. The copper nanoparticles will be formed after 2-3 hours.

4.4.2 Zinc nanoparticles

The stock solution is prepared by dissolving 2.87g of Zinc sulphate (ZnSO₄) in 100ml of DH₂O. Add 9ml of the 100mM ZnSO₄ solution to 1ml of the leaf extract and is allowed to react in room temperature. The Zinc nanoparticles will be formed after 2-3 hours.

4.5 Test microorganisms

Fusarium oxysporum cubense is a fungal plant pathogen that causes Panama disease of banana (Musa spp.), also known as fusarium wilt of banana. The test organism were obtained

from The department of Pathology, Indian Council of Agricultural Research (ICAR), New Delhi.

4.6 Solvents

4.6.1 Distilled water

The water that has been boiled into vapour and condensed back into liquid in a separate container. Impurities in the original water that do not boil below or at the boiling point of water remain in the original container. Thus, distilled water is one type of purified water. It goes through a distillation process.

4.6.2 Propane

It is a three-carbon alkane with the molecular formula C₃H₈. Its boiling point is -42°C and its melting point is-188°C and has a molecular mass of 44.1 g/mol.

4.6.3 Hexane

It is an alkane of six carbon atoms, with the chemical formula C₆H₁₄. They are colorless liquids, odorless when pure, with boiling points between 50 and 70 °C (122 and 158 °F). It is a good solvent if trying to dissolve a non-polar compound .It is highly flammable and its vapours can be explosive.

4.6.4 Acetone

It is an organic compound with the formula (CH₃)₂CO. It is a colorless, volatile, flammable liquid and is the simplest and smallest ketone. It is a good solvent for many plastics and some synthetic fibers and used for thinning polyester resin, cleaning tools etc. It is used as one of the volatile components of some paints and varnishes.

4.6.5 Methanol

It is the simplest alcohol, consisting of a methyl group linked to a hydroxyl group. It is a light, volatile, colorless, flammable liquid with a distinctive odour. It is however far more toxic than ethanol. At room temperature, it is a polar liquid. It can be used as an antifreeze, solvent, fuel, and as a denaturant for ethanol.

4.7 Characterization of nanoparticles

4.7.1 UV-Vis spectroscopy

The periodic scans of the optical absorbance between 345 and 700 nm with a UV- Vis spectrophotometer (Model 118, Systronics, Mumbai, India) at a resolution of 1 nm were performed to investigate the reduction rate of green synthesised nanoparticles. Deionised water was used to adjust the baseline.

The reduction of Cu²⁺ and Zn²⁺ was monitored periodically by using a UV- Vis Spectrophotometer and the UV- Vis spectra of the reaction solutions were measured in the range of 375–760 nm.

4.7.2 SEM-EDX analysis

SEM-EDX Analysis was carried out in instrument JSM 6390 with acceleration voltage 20 kV. SEM reveals information about the sample including external morphology, chemical composition and crystalline structure and orientation of materials making up the sample. SEM provides detailed high-resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back scattered electron signal. The EDX spectrum of the silver nanoparticles was performed to confirm the presence of elemental silver signal and provides quantitative compositional information.

4.8 Antifungal assay

Antifungal assay was performed by agar well diffusion method. Active cultures of *Fusarium oxysporum cubense* were aseptically swabbed on Potato Dextrose Agar (PDA) plates using sterile cotton swabs. Wells of 7 mm diameter were made in the inoculated plates using sterile syringe (with front end cut and polished) and wells are filled with 50, 100 and 150 μ l of nanoparticle solution, control (stock solution) and sample (fresh and dry leaf extracts). The plates were

incubated at 25°C for 72 hours after which the diameter of zones of inhibition were measured on regular intervals (24, 48 and 72 hrs).

4.9 Statistical analysis

The results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA)

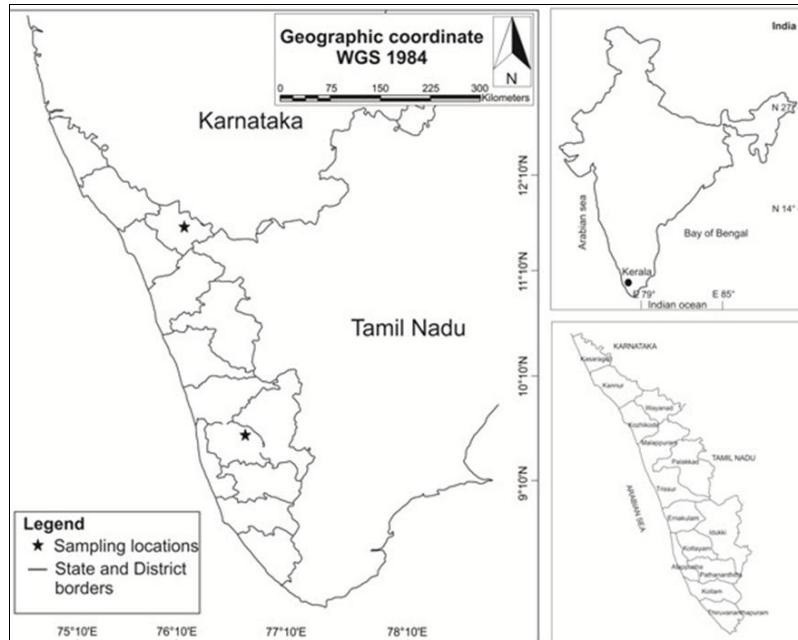


Fig 1: Map of Kerala showing the sample collection point.



Fig 2: Description of Communist palla (*Chromolaena odorata*) a) plant in natural habitat, b) mature seeds in the plant, c), d) and e) plant with flowers and leaves. Photo courtesy: Wikipedia.



Fig 3: Adalodakam (*Justicia adhatoda*), description a) plant with flowers, b) young leaves, c) and d) young plant with flowers. Photo courtesy: Wikipedia

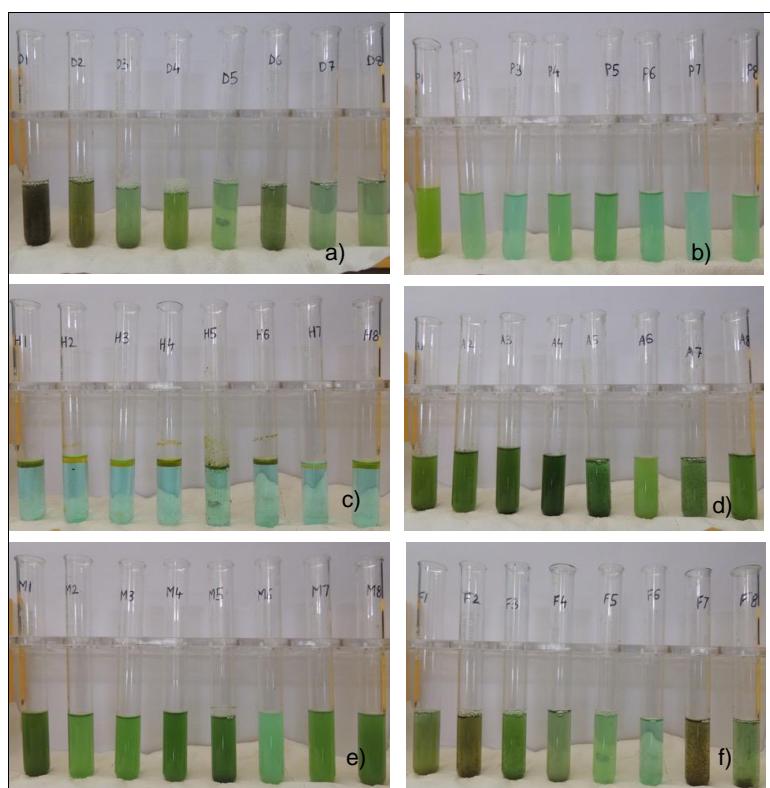


Fig 4: Green synthesised Copper nanoparticles of *Chromolaena odorata*, *Justicia adhatoda*, a) dry leaf distilled water extracts, b) dry leaf propane extracts, c) dry leaf hexane extracts, d) dry leaf acetone extracts, e) dry leaf methanol extracts, f) fresh leaf distilled water extracts.

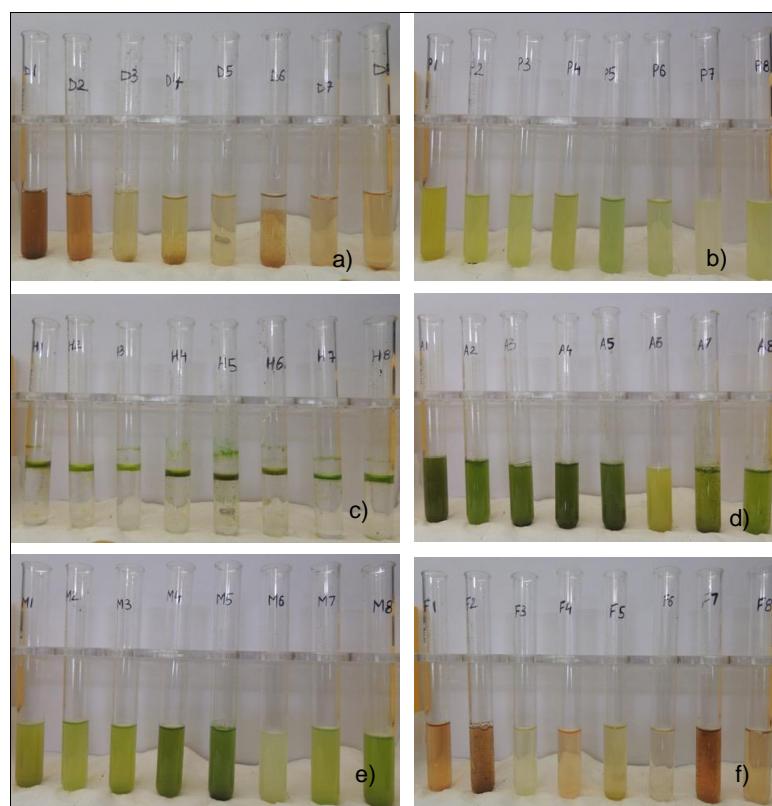


Fig 5: Green synthesised Zinc nanoparticles of *Chromolaena odorata* and *Justicia adhatoda*, a) dry leaf distilled water extracts, b) dry leaf propane extracts, c) dry leaf hexane extracts, d) dry leaf acetone extracts, e) dry leaf methanol extracts, f) fresh leaf distilled water extracts.

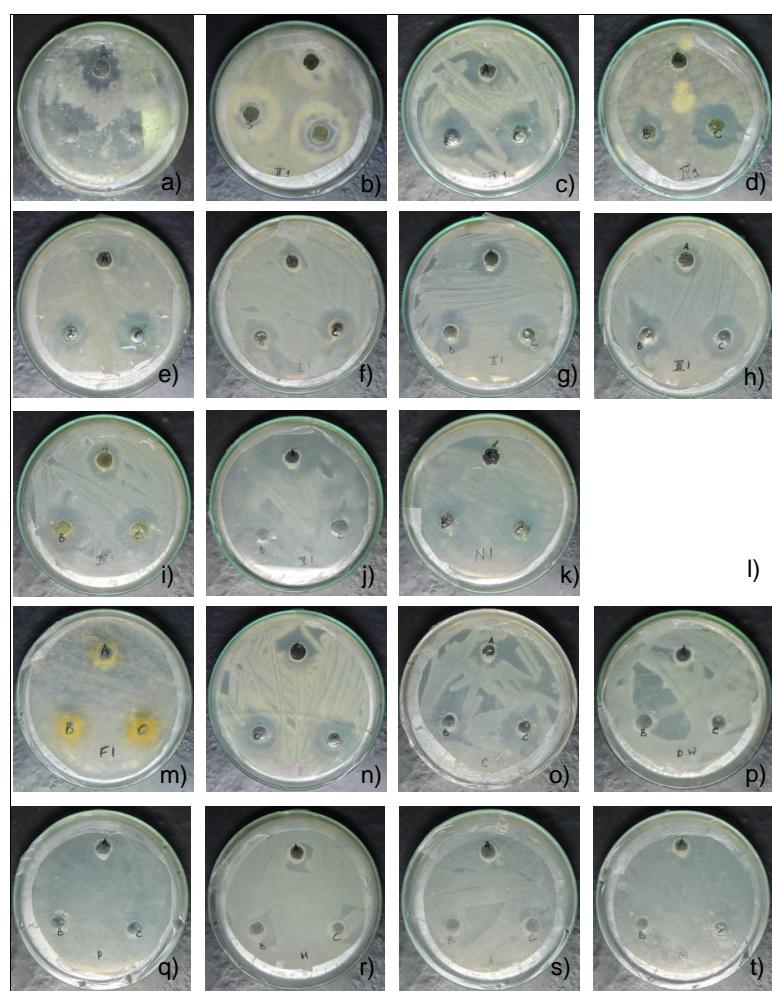


Fig 6: Antifungal activity study using well diffusion method of Communist palla (*Chromolaena odorata*) 24 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test plate in same order for Zn nanoparticles, k) fresh extract nanoparticle (Cu), l) fresh extract nanoparticle (Zn), m) fresh extract alone, n) Cu control plate, o) Zn control plate, p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

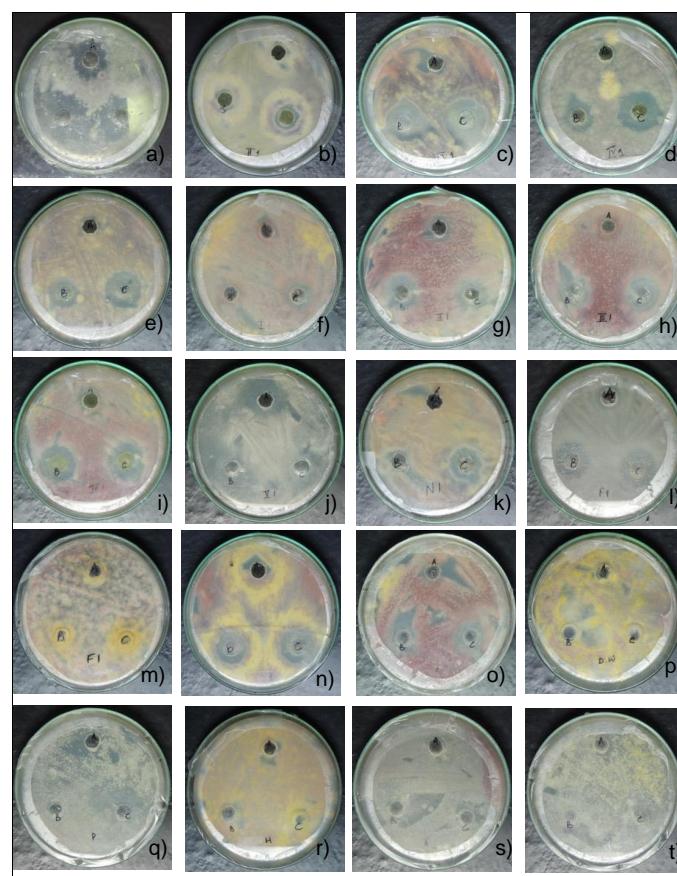


Fig 7: Antifungal activity study using well diffusion method of Communist palla (*Chromolaena odorata*) 48 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test plate in same order for Zn nanoparticles, k) fresh extract nanoparticle (Cu), l) fresh extract nanoparticle (Zn), m) fresh extract alone, n) Cu control plate, o) Zn control plate, p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

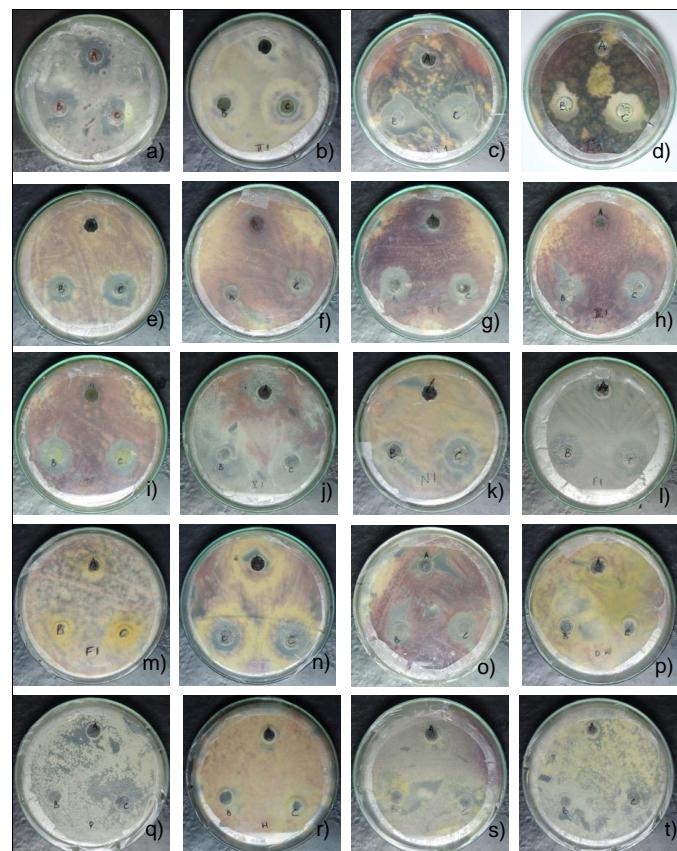


Fig 8: Antifungal activity study using well diffusion method of Communist palla (*Chromolaena odorata*) 72 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test plate in same order for Zn nanoparticles, k) fresh extract nanoparticle (Cu), l) fresh extract nanoparticle (Zn), m) fresh extract alone, n) Cu control plate, o) Zn control plate, p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

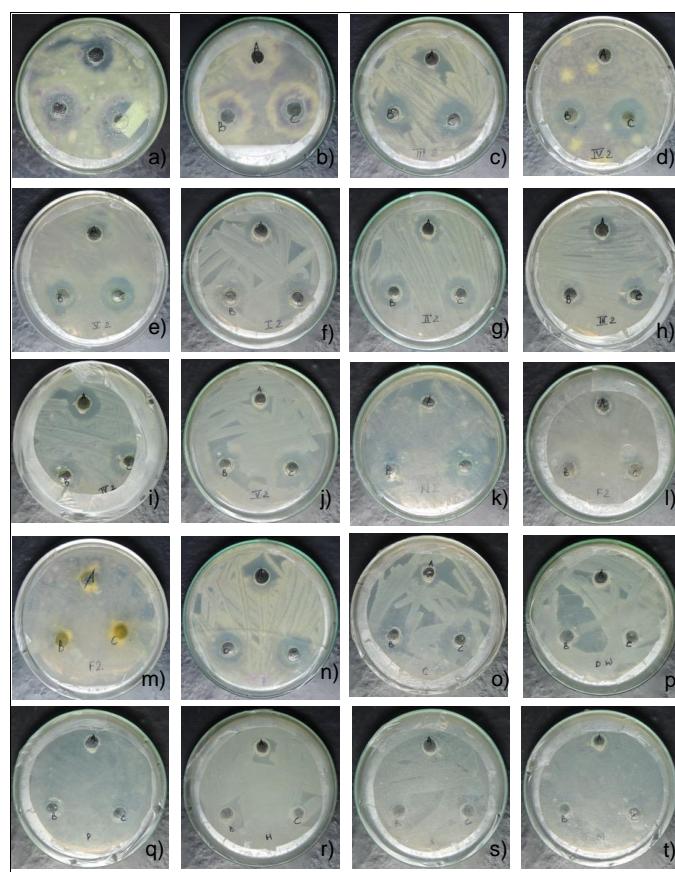


Fig 9: Antifungal activity study using well diffusion method of Adalodakam (*Justicia adhatoda*) 24 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test plate in same order for Zn nanoparticles, k) fresh extract nanoparticle (Cu), l) fresh extract nanoparticle (Zn), m) fresh extract alone, n) Cu control plate, o) Zn control plate, p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

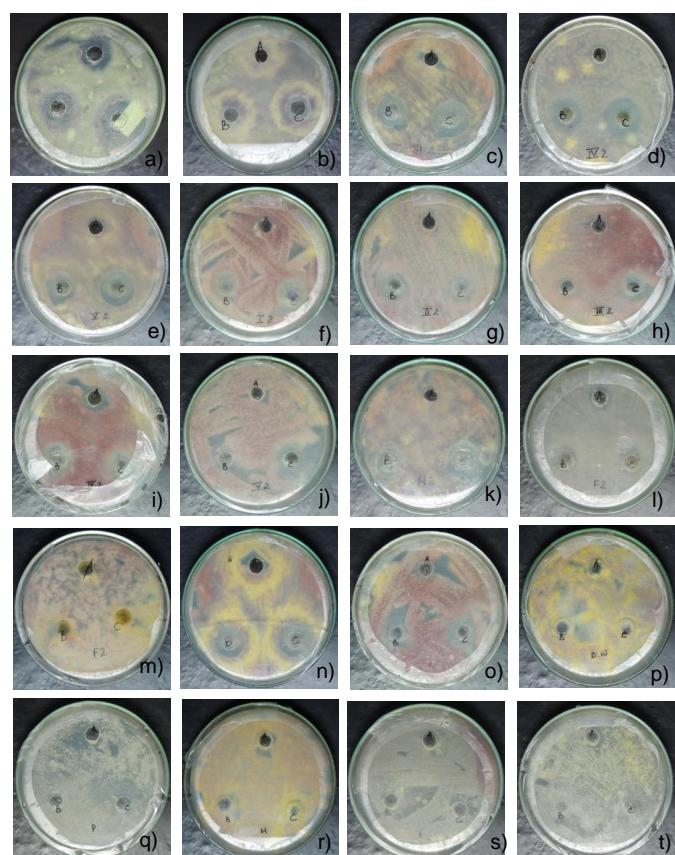


Fig 10: Antifungal activity study using well diffusion method of Adalodakam (*Justicia adhatoda*) 48 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test plate in same order for Zn nanoparticles, k) fresh extract nanoparticle (Cu), l) fresh extract nanoparticle (Zn), m) fresh extract alone, n) Cu control plate, o) Zn control plate, p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

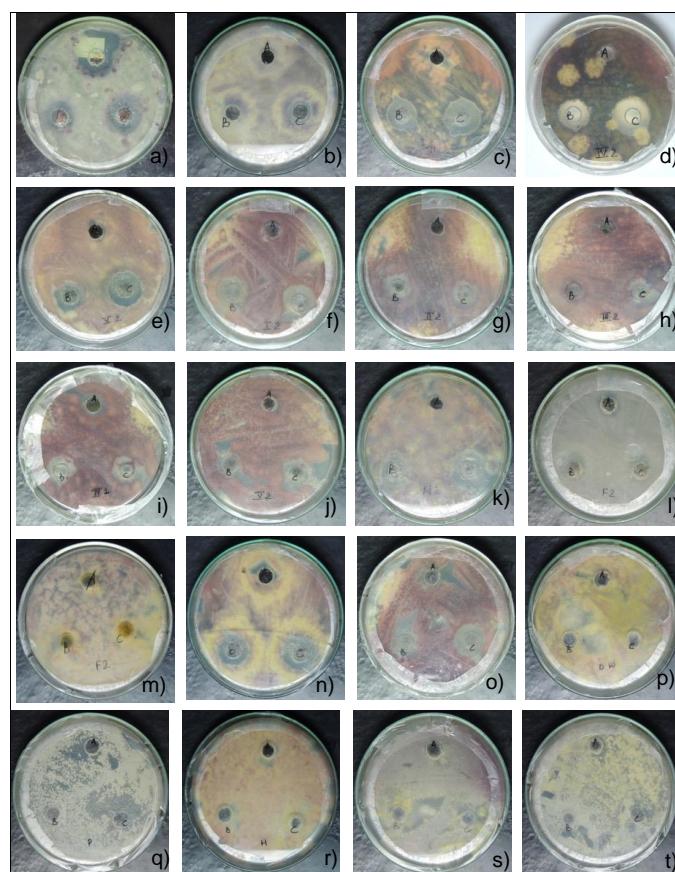


Fig 11: Antifungal activity study using well diffusion method of Adalodakam (*Justicia adhatoda*) 72 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test plate in same order for Zn nanoparticles, k) fresh extract nanoparticle (Cu), l) fresh extract nanoparticle (Zn), m) fresh extract alone, n) Cu control plate, o) Zn control plate, p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

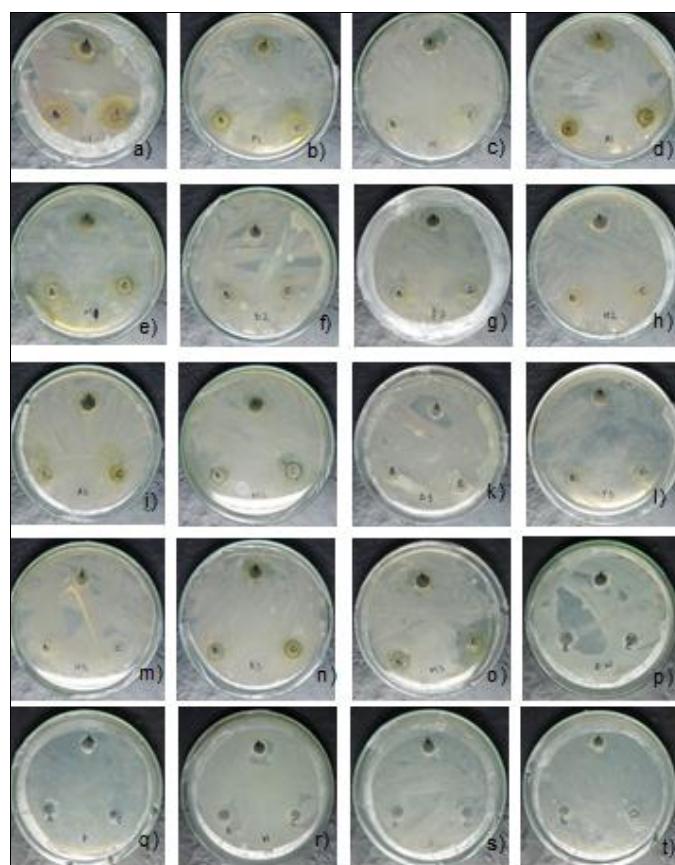


Fig 12: Antifungal activity study using well diffusion method of Communistpalla, (*Chromolaena odorata*) 24 hrs leaf dry extract a) control plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned control plate in same order for Adalodakam (*Justicia adhatoda*), k) to o) above mentioned control plate in same order for Pannal (*Glycosmis pentaphylla*), p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

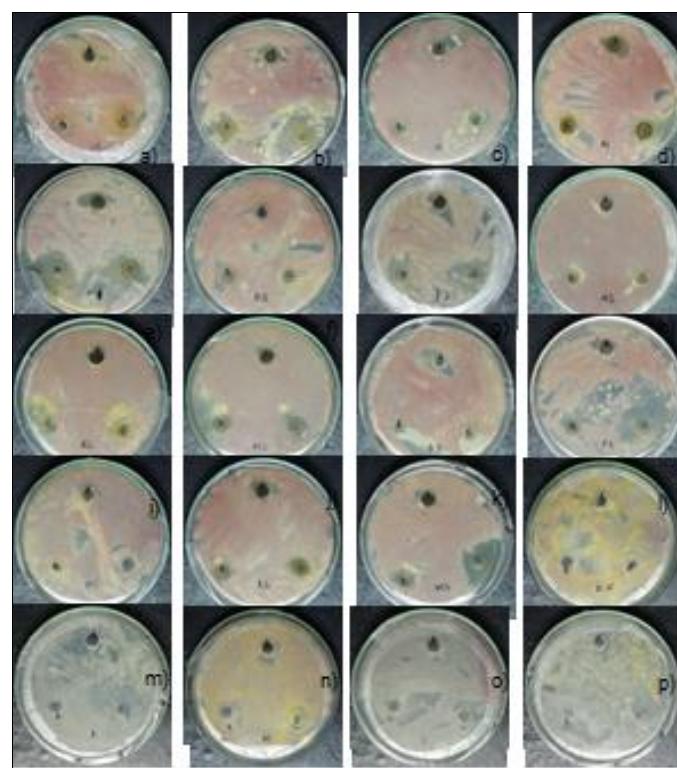


Fig 13: Antifungal activity study using well diffusion method of Communinstpalla, (*Chromolaena odorata*) 48 hrs leaf dry extract a) control plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned control plate in same order for Adalodakam (*Justicia adhatoda*), k) to o) above mentioned control plate in same order for Pannal (*Glycosmis pentaphylla*), p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

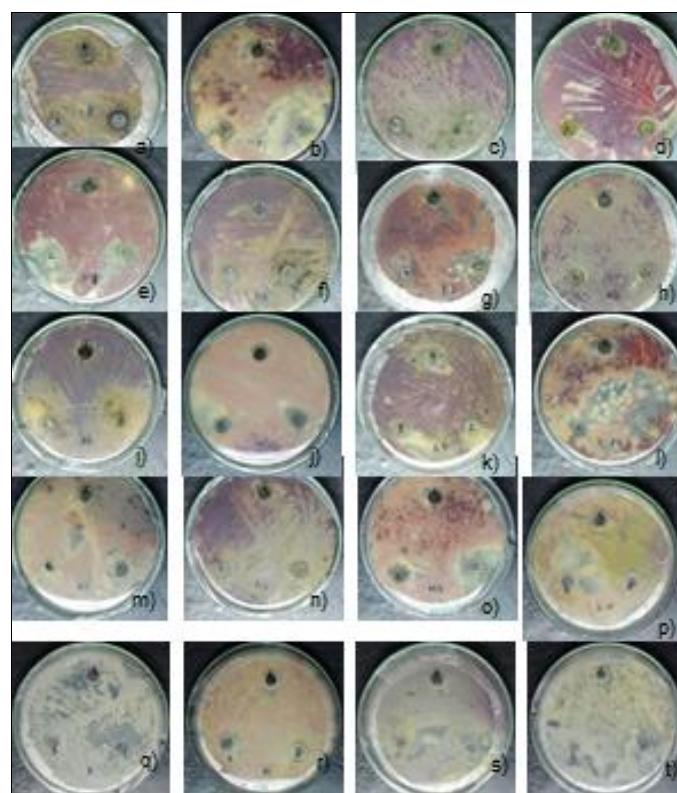


Fig 14: Antifungal activity study using well diffusion method of Communinstpalla, (*Chromolaena odorata*) 72 hrs leaf dry extract a) control plate (Distilled water; 50, 100 and 150 μ l), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned control plate in same order for Adalodakam (*Justicia adhatoda*), k) to o) above mentioned control plate in same order for Pannal (*Glycosmis pentaphylla*), p) to t) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

Table 1: Zone of inhibition (24 hrs) against *Fusarium oxysporum* cubense by the Distilled Water extract (50, 100 and 150 µl) of various plant leaves (*Chromolaena odorata*, *Justicia adhatoda*). Control; CuSO₄ and ZnSO₄ solution, Sample; dry leaf extract in distilled water.

Plant name	Nanoparticles	Control			Sample			Test		
		Measure of zone of inhibition (cm)			Measure of zone of inhibition (cm)			Measure of zone of inhibition (cm)		
		50	100	150	50	100	150	50	100	150
Communist Palla (Distilled Water)	Copper	1.7	2.5	3	-	-	-	2.5	3	3
	Zinc	1.4	1.5	2	-	-	-	1.5	1.8	2.3
Aadalodakam (Distilled Water)	Copper	1.7	2.5	3	-	-	-	2	2.1	3.2
	Zinc	1.4	1.5	2	-	-	-	1.7	2	2.2
Communist Palla (Proponic Extract)	Copper	1.7	2.5	3	1.8	2.2	2.3	2	2.5	3.2
	Zinc	1.4	1.5	2	1.8	2.2	2.3	1.8	2.5	2.3
Aadalodakam (Proponic Extract)	Copper	1.7	2.5	3	1.6	1.7	2	2	2.4	3.1
	Zinc	1.4	1.5	2	1.6	1.7	2	1.5	2	2.3
Communist Palla (Hexonic Extract)	Copper	1.7	2.5	3	-	-	1.5	2	2.5	2.7
	Zinc	1.4	1.5	2	-	-	1.5	1.5	2.2	2.5
Aadalodakam (Hexonic Extract)	Copper	1.7	2.5	3	-	-	-	1.7	2	2.5
	Zinc	1.4	1.5	2	-	-	-	1.8	1.9	2.1
Communist Palla (Acetonic Extract)	Copper	1.7	2.5	3	1.8	2	2.1	-	2.2	2.5
	Zinc	1.4	1.5	2	1.8	2	2.1	1.8	2.6	2.8
Aadalodakam (Acetonic Extract)	Copper	1.7	2.5	3	1.2	1.5	1.5	-	1.8	2.5
	Zinc	1.4	1.5	2	1.2	1.5	1.5	2	2.2	2.3
Communist Palla (Methanonic Extract)	Copper	1.7	2.5	3	2	2.2	2.3	1.3	2	2.3
	Zinc	1.4	1.5	2	2	2.2	2.3	2	2.2	2.3
Aadalodakam (Methanonic Extract)	Copper	1.7	2.5	3	1.5	1.9	2	1.4	2.3	2.4
	Zinc	1.4	1.5	2	1.5	1.9	2	1.5	1.9	2

Table 2: Zone of inhibition (48 hrs) against *Fusarium oxysporum* cubense by the Distilled Water extract (50, 100 and 150 µl) of various plant leaves (*Chromolaena odorata*, *Justicia adhatoda*). Control; CuSO₄ and ZnSO₄ solution, Sample; dry leaf extract in distilled water.

Plant name	Nanoparticles	Control			Sample			Test		
		Measure of zone of inhibition (cm)			Measure of zone of inhibition (cm)			Measure of zone of inhibition (cm)		
		50	100	150	50	100	150	50	100	150
Communist Palla (Distilled Water)	Copper	1.6	2.1	2.7	-	-	-	2.4	2.7	2.7
	Zinc	1.3	1.4	1.8	-	-	-	-	-	1.8
Aadalodakam (Distilled Water)	Copper	1.6	2.1	2.7	-	-	-	1.9	2.2	3
	Zinc	1.3	1.4	1.8	-	-	-	1.3	1.5	1.7
Communist Palla (Proponic Extract)	Copper	1.6	2.1	2.7	2	2.1	3.1	1	1.3	1.6
	Zinc	1.3	1.4	1.8	2	2.1	3.1	1.6	2.3	2
Aadalodakam (Proponic Extract)	Copper	1.6	2.1	2.7	1.6	2	2.5	-	-	1.3
	Zinc	1.3	1.4	1.8	1.6	2	2.5	-	1.8	1.9
Communist Palla (Hexonic Extract)	Copper	1.6	2.1	2.7	1.2	-	1.5	1.5	2.7	2.9
	Zinc	1.3	1.4	1.8	1.2	-	1.5	-	1.9	2.1
Aadalodakam (Hexonic Extract)	Copper	1.6	2.1	2.7	-	-	-	1.3	2	2.5
	Zinc	1.3	1.4	1.8	-	-	-	-	1.7	1.8
Communist Palla (Acetonic Extract)	Copper	1.6	2.1	2.7	1.7	2	1.7	-	2	2.5
	Zinc	1.3	1.4	1.8	1.7	2	1.7	1.6	2.2	2.8
Aadalodakam (Acetonic Extract)	Copper	1.6	2.1	2.7	-	1.5	-	-	1.7	2.5
	Zinc	1.3	1.4	1.8	-	1.5	-	1.5	1.9	2.3
Communist Palla (Methanonic Extract)	Copper	1.6	2.1	2.7	1.7	2.5	2.8	1.7	1.8	2.2
	Zinc	1.3	1.4	1.8	1.7	2.5	2.8	1.7	1.9	2
Aadalodakam (Methanonic Extract)	Copper	1.6	2.1	2.7	1.3	1.7	2	-	1.8	2.3
	Zinc	1.3	1.4	1.8	1.3	1.7	2	1.3	1.6	2.1

Table 3: Zone of inhibition (72 hrs) against *Fusarium oxysporum* cubense by the Distilled Water extract (50, 100 and 150 µl) of various plant leaves (*Chromolaena odorata*, *Justicia adhatoda*). Control; CuSO₄ and ZnSO₄ solution, Sample; dry leaf extract in distilled water.

Plant name	Nanoparticles	Control			Sample			Test		
		Measure of zone of inhibition (cm)			Measure of zone of inhibition (cm)			Measure of zone of inhibition (cm)		
		50	100	150	50	100	150	50	100	150
Communist Palla (Distilled Water)	Copper	1.4	2	2.5	-	-	-	2.4	2.5	2.6
	Zinc	1.3	1.5	1.8	-	-	-	-	-	1.7
Aadalodakam (Distilled Water)	Copper	1.4	2	2.5	-	-	-	1.8	2.2	2.5
	Zinc	1.3	1.5	1.8	-	-	-	-	1.5	1.8

Communist Palla (Proponic Extract)	Copper	1.4	2	2.5	1.4	1.5	1.9	-	1.2	1.5
	Zinc	1.3	1.5	1.8	1.4	1.5	1.9	1.6	1.9	2
Aadalodakam (Proponic Extract)	Copper	1.4	2	2.5	1.5	1.4	1.7	-	-	1.2
	Zinc	1.3	1.5	1.8	1.5	1.4	1.7	-	1.6	1.7
Communist Palla (Hexonic Extract)	Copper	1.4	2	2.5	-	-	1.4	1.8	2.2	2.7
	Zinc	1.3	1.5	1.8	-	-	1.4	-	1.7	2
Aadalodakam (Hexonic Extract)	Copper	1.4	2	2.5	-	-	-	1.5	2	2.3
	Zinc	1.3	1.5	1.8	-	-	-	-	1.3	1.7
Communist Palla (Acetonic Extract)	Copper	1.4	2	2.5	1.5	1.8	1.5	-	2	2.4
	Zinc	1.3	1.5	1.8	1.5	1.8	1.5	-	2.1	2.2
Aadalodakam (Acetonic Extract)	Copper	1.4	2	2.5	-	1.3	-	-	1.7	2.2
	Zinc	1.3	1.5	1.8	-	1.3	-	1.3	1.7	1.9
Communist Palla (Methanonic Extract)	Copper	1.4	2	2.5	1.6	2.3	2.5	-	1.7	2.1
	Zinc	1.3	1.5	1.8	1.6	2.3	2.5	1.6	1.7	1.8
Aadalodakam (Methanonic Extract)	Copper	1.4	2	2.5	-	-	2	-	1.7	2.2
	Zinc	1.3	1.5	1.8	-	-	2	1.3	1.5	1.7

Table 4: Zone of inhibition against *Fusarium oxysporum* cubense by the various solvents (Distilled water, Propanol, Hexane, Acetone, Methanol) in 50, 100 and 150 µl volume during 24, 48 and 72 hrs of incubation period.

No	Control solvents	Measure of zone of inhibition (cm), 24 hrs			Measure of zone of inhibition (cm), 48 hrs			Measure of zone of inhibition (cm), 72 hrs		
		50	100	150	50	100	150	50	100	150
1	Distilled water	-	-	-	-	-	-	-	-	-
2	Propanol	1.7	-	2.1	1.3	-	2	-	-	1.5
3	Hexane	1.6	-	-	-	-	-	-	-	-
4	Acetone	-	-	1.7	-	-	1.5	-	-	1.4
5	Methanol	1.4	1.5	1.7	-	-	1.5	-	-	1.3

Table 5: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Distilled water extract of Communist Palla (*Chromolaena odorata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	435nm	385nm	560nm	435nm	680nm	560nm
½ hr	1.530	0.142	0.633	0.130	0.421	0.103
1 hr	1.556	0.163	0.674	0.151	0.458	0.146
1 ½ hr	1.728	0.197	0.701	0.186	0.497	0.159
2 hr	1.743	0.238	0.725	0.204	0.526	0.194
2 ½ hr	1.754	0.257	0.759	0.227	0.531	0.213

Table 6: UV Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Distilled water extract of Aadalodakam (*Justicia adhatoda*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	435nm	385nm	560nm	435nm	680nm	560nm
½ hr	1.642	0.174	0.570	0.157	0.314	0.162
1 hr	1.658	0.182	0.584	0.190	0.332	0.187
1 ½ hr	1.671	0.197	0.597	0.234	0.350	0.219
2 hr	1.684	0.206	0.606	0.255	0.368	0.243
2 ½ hr	1.693	0.231	0.623	0.267	0.375	0.258

Table 7: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Propane extract of Communist Palla (*Chromolaena odorata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	435nm	385nm	560nm	435nm	680nm	560nm
½ hr	1.542	0.015	1.624	0.024	1.357	0.020
1 hr	1.598	0.037	1.635	0.046	1.501	0.039
1 ½ hr	1.603	0.058	1.671	0.059	1.523	0.052
2 hr	1.624	0.072	1.689	0.073	1.558	0.072
2 ½ hr	1.655	0.064	1.716	0.068	1.590	0.065

Table 8: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Propane extract of Aadalodakam (*Justicia adhatoda*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	435nm	385nm	560nm	435nm	680nm	560nm
½ hr	0.786	0.009	0.528	0.015	0.601	0.003
1 hr	0.793	0.028	0.567	0.043	0.624	0.014
1 ½ hr	0.815	0.064	0.512	0.076	0.671	0.031
2 hr	0.836	0.059	0.574	0.061	0.693	0.054
2 ½ hr	0.852	0.052	0.601	0.052	0.726	0.046

Table 9: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Hexane extract of Communist Palla (*Chromolaena odorata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	435nm	385nm	560nm	435nm	680nm	560nm
½ hr	0.065	0.004	0.031	0.005	0.020	0.007
1 hr	0.098	0.016	0.054	0.010	0.034	0.012
1 ½ hr	0.103	0.025	0.072	0.023	0.055	0.019
2 hr	0.142	0.034	0.093	0.037	0.059	0.025
2 ½ hr	0.149	0.031	0.113	0.035	0.062	0.031

Table 10: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Hexane extract of Aadalodakam (*Justicia adhatoda*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	435nm	385nm	560nm	435nm	680nm	560nm
½ hr	0.094	0.234	0.024	0.222	0.022	0.196
1 hr	0.106	0.265	0.036	0.247	0.040	0.218
1 ½ hr	0.113	0.283	0.051	0.281	0.057	0.237
2 hr	0.127	0.305	0.079	0.314	0.071	0.244
2 ½ hr	0.135	0.297	0.084	0.301	0.078	0.251

Table 11: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Acetone extract of Communist Palla (*Chromolaena odorata*) leaves during different time of incubation.

Time	Copper 435nm	Zinc 385nm	Copper 560nm	Zinc 435nm	Copper 680nm	Zinc 560nm
½ hr	1.624	0.753	1.546	0.724	1.487	0.642
1 hr	1.691	0.786	1.582	0.744	1.494	0.681
1 ½ hr	1.748	0.791	1.604	0.768	1.523	0.756
2 hr	1.773	0.824	1.649	0.799	1.684	0.791
2 ½ hr	1.790	0.831	1.673	0.823	1.697	0.800

Table 12: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Acetone extract of Aadalodakam (*Justicia adhatoda*) leaves during different time of incubation.

Time	Copper 435nm	Zinc 385nm	Copper 560nm	Zinc 435nm	Copper 680nm	Zinc 560nm
½ hr	0.846	0.324	0.948	0.318	0.971	0.401
1 hr	0.866	0.357	0.973	0.334	0.998	0.428
1 ½ hr	0.901	0.397	1.152	0.375	1.126	0.452
2 hr	0.954	0.425	1.179	0.399	1.149	0.461
2 ½ hr	0.962	0.447	1.192	0.416	1.155	0.441

Table 13: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Methanol extract of Communist Palla (*Chromolaena odorata*) leaves during different time of incubation.

Time	Copper 435nm	Zinc 385nm	Copper 560nm	Zinc 435nm	Copper 680nm	Zinc 560nm
½ hr	0.597	0.753	0.621	0.771	0.634	0.802
1 hr	0.634	0.768	0.637	0.796	0.658	0.834
1 ½ hr	0.659	0.791	0.658	0.816	0.681	0.822
2 hr	0.670	0.824	0.673	0.833	0.703	0.818
2 ½ hr	0.702	0.814	0.694	0.837	0.722	0.824

Table 14: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Methanol extract of Aadalodakam (*Justicia adhatoda*) leaves during different time of incubation.

Time	Copper 435nm	Zinc 385nm	Copper 560nm	Zinc 435nm	Copper 680nm	Zinc 560nm
½ hr	0.816	0.647	0.834	0.681	0.854	0.594
1 hr	0.837	0.673	0.861	0.694	0.868	0.621
1 ½ hr	0.869	0.694	0.872	0.726	0.880	0.638
2 hr	0.916	0.713	0.896	0.743	0.893	0.644
2 ½ hr	0.937	0.728	0.924	0.759	0.931	0.662

Table 15: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Fresh extract of Communist Palla (*Chromolaena odorata*) leaves during different time of incubation.

Time	Copper 435nm	Zinc 385nm	Copper 560nm	Zinc 435nm	Copper 680nm	Zinc 560nm
½ hr	0.042	0.009	0.056	0.005	0.064	0.004
1 hr	0.049	0.018	0.068	0.013	0.072	0.016
1 ½ hr	0.067	0.034	0.079	0.028	0.081	0.027
2 hr	0.072	0.027	0.062	0.041	0.077	0.038
2 ½ hr	0.094	0.025	0.072	0.035	0.082	0.034

Table 16: Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Fresh extract of Aadalodakam (*Justicia adhatoda*) leaves during different time of incubation.

Time	Copper 435nm	Zinc 385nm	Copper 560nm	Zinc 435nm	Copper 680nm	Zinc 560nm
½ hr	0.058	0.007	0.084	0.010	0.074	0.008
1 hr	0.067	0.011	0.097	0.105	0.082	0.112
1 ½ hr	0.073	0.019	0.103	0.127	0.094	0.119
2 hr	0.094	0.025	0.099	0.116	0.099	0.124
2 ½ hr	0.102	0.027	0.081	0.113	0.111	0.126

5. Results and discussion

5.1 Synthesis of nanoparticles

Nanoparticles were synthesized from the leaf extracts of *Chromolaena odorata* and *Justicia adhatoda* using solvents like distilled water, propane, hexane, methanol and acetone.

5.1.1 Copper nanoparticles

Copper nanoparticles were synthesized from leaf extract of different plants (*Chromolaena odorata*, *Justicia adhatoda*). Leaf extract was added to 100Mm copper sulphate solution and kept to reaction to take place. A colour change was observed from blue to pale yellow. This occurred due to the reduction of copper ions present in the solution

5.1.2 Zinc nanoparticles

Zinc nanoparticles were synthesized from leaf extract of different plants (*Chromolaena odorata*, *Justicia adhatoda*). Leaf extract was added to 100Mm zinc sulphate solution and kept to reaction to take place. A colour change was observed from colourless to pale brown. This occurred due to the reduction of zinc ions present in the solution.

5.2 Characterization of nanoparticles

5.2.1 Copper nanoparticles-UV spectroscopy

Synthesized copper nanoparticles were characterized by UV-VIS spectrophotometry. The maximum peak was found to be at 435 nm for the leaf extracts of *Chromolaena odorata*, *Justicia adhatoda* with distilled water and propane and 680nm with hexane, acetone and methanol solvent extracts. The intensity of the peak was increased with time until the reduction completes.

5.2.2 Zinc nanoparticles-UV spectroscopy

Synthesized zinc nanoparticles were characterized by UV-VIS spectrophotometry. The maximum peak was found to be at 385nm for the leaf extracts of *Chromolaena odorata*, *Justicia adhatoda* with each solvent.

The SEM-XRD analysis proved the effective formation of copper and zinc nanoparticles in all the samples.

5.3 Antifungal assay

The leaf extracts of *Chromolaena odorata*, *Justicia adhatoda*, with solvents distilled water, propane, hexane, methanol and acetone and the fresh extract showed the growth inhibitory effects against *Fusarium oxysporum cubense*.

5.3.1 Communist palla (*Chromolaena odorata*)

The copper nanoparticles formed from the leaf extracts with solvents distilled water, propane and hexane had a better zone of inhibition than the zinc nanoparticles. However the extract with acetone and methanol forming zinc particles had a better zone of inhibition than copper nanoparticles. The highest inhibitory zone was showed by the Cu nanoparticles formed from distilled water extract and its action remained for more than 72 hours.

5.3.2 Aadalodakam (*Justicia adhatoda*)

The antifungal activity with both zinc and copper nanoparticles with the various solvents showed a similar range of inhibition. The propane and distilled water extracts had a greater result than compared to others.

6. Conclusions

The results shows that leaf extracts of *Chromolaena odorata*, *Justicia adhatoda*, with solvents distilled water, propane,

hexane, methanol and acetone and the fresh extract are used for the synthesis of copper and zinc nanoparticles. Copper and zinc nanoparticle shows greater antifungal activity than copper sulphate and zinc sulphate, respectively and leaf extract. The maximum zone of inhibition was at 150 µl for all the bacterial cultures. It indicates that the zone of inhibition increases with as the concentration of nanoparticles increased. An overall result showed that the synthesized nanoparticles could be used as a remedy against the Panama wilt disease.

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