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Green Synthesis of Copper and Zinc Nanoparticles from *Ocimum tenuiflorum* and *Tabernaemontana divaricata* and Evaluation of their Antifungal Activity against *Fusarium oxysporum cubense*

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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 *Ocimum tenuiflorum* and *Tabernaemontana divaricata*. 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 150µl 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.

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Keywords

Nanoparticles, *Fusarium oxysporum cubense, Panama wilt, Ocimum tenuiflorum.*

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). Nano-technology mainly consists of the processing of, separation, consolidation, and deformation of materials by one atom or one molecule (Prasad *et al.*, 2008). Nanoparticles has 1-100 nm in size and they possess novel physical and chemical properties (Sajeshkumar *et al.*, 2015a; Sajeshkumar *et al.*, 2015b). Nanoparticles bear antibacterial properties (Hajipour *et*

al., 2012). 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). 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. 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).

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). 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).

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). 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) 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).

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). 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).

Application of nanoparticles

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

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).

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). 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.

Zinc nanoparticles

Zinc nanoparticles have wide application; various synthetic methods have been employed to produce ZnNps (Chen *et al.*, 2007). Zinc nanoparticles can produced from zinc oxide and zinc sulphate. Zinc nanoparticles has several medicinal uses, which harm skin, stomach, intestine and lymphastic 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).

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). 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.

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 tropical chlormidazole in1958 and the subsequent introduction of IV AmB in 1960.

Antifungal creams, liquids and sprays are used to treat fungal infections.

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.

Objectives

Synthesis of copper and zinc nanoparticles using leaf extracts of eight different plants; Tulsi (*Ocimum tenuiflorum*) and Nandhyar vattam (*Tabernaemontana divaricata*) and determine the antifungal properties of these nanoparticles against *Fusarium oxysporum cubense*.

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.

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).

Ocimum tenuiflorum (Holy basil; Tulsi)

Ocimum tenuiflorum is an aromatic shrub, commonly known as 'The Queen of Herbs' and is used for medicinal and spiritual purposes. Ancient ayurvedic suggests that tulsi is a tonic for the body, mind and spirit and in modern days it provides solutions to many health problems. Tulsi has various benefits which includes giving beauty, intelligence, stamina, sweetness to voice and even other medicinal benefits like cough, asthma, diarrhea, fever, eye diseases, indigestion, gastric and cardiac disorders, back pain, skin diseases, insect and snake bites and malaria.

It has anti-bacterial, anti-viral and anti-fungal activity that includes activity against many pathogens responsible for human infections. It also boost defenses against infective threats by enhancing immune responses in humans. They are also used as a herbal mouth wash for treating bad breath, gum disease and mouth ulcers. They have both antibacterial antioxidant and anti-inflammatory functions which make them useful in healing wounds. (Cohen, 2014).

Taxonomical classification (*Ocimum tenuiflorum*; Tulsi)

Kingdom: Plantae-- planta, plantes, plants, vegetal

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: *Ocimum tenuiflorum*

Tabernaemontana divaricata (Tagor; Crepe jasmine; Nandhyar vattam)

Plants are well known as a major source of modern medicines and Tabernaemontana is one such plant used in Ayurvedic, Chinese and Thai traditional medicines for the treatment of various diseases and fever.

They are found in tropical areas as a green plant and usually has a sweet-scented double flowers. It is distributed in countries like Brazil, Egypt, India, Sri Lanka, Vietnam, Malaysia and Thailand. It is commomly known as Togor, Dudhphul in Bangladesh and Wax flower, Crepe flower, Crepe jasmine in India. The plant generally grows to a height of 5–6 feet (1.5–1.8 m) and is dichotomously-branched. Its extracts are frequently used

in India for the treatment of variety of ailments and for infectious diseases. It acts as a natural antioxidant, has anti-microbial activities and even has cytotoxic capacities. (Pratchayasakul *et al.*, 2008)

Taxonomical classification (*Tabernaemontana divaricata*; Tagor)

Kingdom: Plantae-- planta, plantes, plants, vegetal

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: Tabernaemontana

Species: *Tabernaemontana divaricata*

Hypothesis

The current research work is based on the following hypothesis

Plant extracts of Tulsi (*Ocimum tenuiflorum*) and Nandhyar vattam (*Tabernaemontana divaricata*) could be used as antifungal agents.

These plant extracts could be used in formulating nanoparticles (copper and zinc) and their antifungal activity vary widely.

Materials and Methods

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). Maximum rainfall occurs from June to September mainly due to South West Monsoon and temperatures are highest in May and November.

Sample collection

Samples of Tulsi (*Ocimum tenuiflorum*) and Nandhyar vattam (*Tabernaemontana divaricata*) 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 60ºC for 48 hrs and later stored in air tight polyethylene zipper bag for analysis.

Extraction method

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.

Fresh extraction

Leaf extract is prepared with 10 g of fresh leafs (*Ocimum tenuiflorum*, *Tabernaemontana divaricata*) 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.

Synthesis of nanoparticles

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.

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.

Test microorganisms

Fusrium 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.

Solvents

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.

Propane

It is a three-carbon alkane with the molecular formula C_3H_8. Its boiling point is -42°C and its melting point is-188°C and has a molecular mass of 44.1 g/mol.

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.

Acetone

It is an organic compound with the formula $(CH_3)_2CO$. 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.

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.

Characterization of nanoparticles

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.

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.

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 µl 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).

Table.1 Different vernacular names of Tulsi (*Ocimum tenuiflorum*) around the globe and India.

Language	Names
Scientific names	*Ocimum tenuiflorum*
Name in various global languages	
French	
German	
English	Holy basil
Name in various Indian languages	
Sanskrit	
Hindi	Tulsi
Urdu	
Marathi	Tulshi
Kannada	
Gujarati	
Malayalam	Thulsi
Tamil	

Table.2 Different vernacular names of Nandhyar vattam (*Tabernaemontana divaricata*) around the globe and India.

Language	Names
Scientific names	*Tabernaemontana divaricata*
Name in various global languages French	
German	
English	Crape jasmin
Name in various Indian languages	
Sanskrit	Nandivrksa
Hindi	Tagar
Urdu	
Marathi	Ananta
Kannada	Nandi battalu
Gujarati	Sagar
Malayalam	Nandhiar vattam
Tamil	Nandhiar vattai

Table.3 Zone of inhibition (24 hrs) against *Fusarium oxysporum* cubense by the Distilled Water extract (50, 100 and 150 µl) of various plant leaves (*Ocimum tenuiflorum, Tabernaemontana divaricata*). 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**		
		50	**100**	**150**	**50**	**100**	**150**	**50**	**100**	**150**
TULSI (Distilled water)	Copper	1.7	2.5	3	-	-	-	2.1	3.2	**3.3**
	Zinc	1.4	1.5	2	-	-	-	1.6	2.2	**2.3**
NANDHYAR VATAM (Distilled water)	Copper	1.7	2.5	3	-	-	-	1.7	2.8	**2.9**
	Zinc	1.4	1.5	2	-	-	-	1.5	2.2	**2.4**
TULSI (Proponic extract)	Copper	1.7	2.5	3	1.5	2	3.3	2	2	**3**
	Zinc	1.4	1.5	2	1.5	2	3.3	1.8	2	**2.4**
NANDHYAR VATAM (Proponic extract)	Copper	1.7	2.5	3	-	-	-	2.4	2.5	**2.7**
	Zinc	1.4	1.5	2	-	-	-	2	2.3	**2.4**
TULSI (Hexonic extract)	Copper	1.7	2.5	3	-	-	-	1.8	2	**2.6**
	Zinc	1.4	1.5	2	-	-	-	1.9	2.4	**2.6**
NANDHYAR VATAM (Hexonic extract)	Copper	1.7	2.5	3	1.8	2	-	1.4	1.8	**2.4**
	Zinc	1.4	1.5	2	1.8	2	-	1.2	2.3	**2.5**
TULSI (Acetonic extract)	Copper	1.7	2.5	3	1.6	-	-	1.5	2.2	**2.6**
	Zinc	1.4	1.5	2	1.6	-	-	2	2.2	**2.5**
NANDHYAR VATAM (Acetonic extract)	Copper	1.7	2.5	3	2	1.7	2.2	2.3	2.5	**3**
	Zinc	1.4	1.5	2	2	1.7	2.2	2	2	**2.4**
TULSI (Methanonic extract)	Copper	1.7	2.5	3	2	2.4	2.8	1.6	2	**2.4**
	Zinc	1.4	1.5	2	2	2.4	2.8	2	2.4	**2.8**
NANDHYAR VATAM (Methanonic extract)	Copper	1.7	2.5	3	2	2.5	2.7	1.6	2.2	**2.3**
	Zinc	**1.4**	**1.5**	**2**	**2**	**2.5**	**2.7**	**2**	**2.5**	2.7

Table.4 Zone of inhibition (48 hrs) against *Fusarium oxysporum* cubense by the Distilled Water extract (50, 100 and 150 µl) of various plant leaves (*Ocimum tenuiflorum, Tabernaemontana divaricata*). 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**		
		50	**100**	**150**	**50**	**100**	**150**	**50**	**100**	**150**
TULSI (Distilled water)	Copper	1.6	2.1	2.7	-	-	-	2	3	**3.1**
	Zinc	1.3	1.4	1.8	-	-	-	-	1.7	**2**
NANDHYAR VATAM (Distilled water)	Copper	1.6	2.1	2.7	-	-	-	1.7	2.5	**2.7**
	Zinc	1.3	1.4	1.8	-	-	-	-	1.8	**1.9**
TULSI (Proponic extract)	Copper	1.6	2.1	2.7	1.7	2	3.5	1.5	1.8	**2.1**
	Zinc	1.3	1.4	1.8	1.7	2	3.5	1.5	2	**2.1**
NANDHYAR VATAM (Proponic extract)	Copper	1.6	2.1	2.7	2	2.5	3	1.6	1.6	**2**
	Zinc	1.3	1.4	1.8	2	2.5	3	2	2.1	**2.1**
TULSI (Hexonic extract)	Copper	1.6	2.1	2.7	1.5	1.7	1.7	1.9	2	**2.8**
	Zinc	1.3	1.4	1.8	1.5	1.7	1.7	1.4	1.9	**2.3**
NANDHYAR VATAM (Hexonic extract)	Copper	1.6	2.1	2.7	-	-	-	1.5	1.9	**2.1**
	Zinc	1.3	1.4	1.8	-	-	-	-	1.7	**2**
TULSI (Acetonic extract)	Copper	1.6	2.1	2.7	1.6	-	1.8	1.4	2	**2.4**
	Zinc	1.3	1.4	1.8	1.6	-	1.8	1.5	1.6	**1.8**
NANDHYAR VATAM (Acetonic extract)	Copper	1.6	2.1	2.7	1.6	1.8	2.3	2.1	2.3	**2.9**
	Zinc	1.3	1.4	1.8	1.6	1.8	2.3	1.7	2.4	**2.5**
TULSI (Methanonic extract)	Copper	1.6	2.1	2.7	1.2	1.7	1.8	1.4	1.9	**2.1**
	Zinc	1.3	1.4	1.8	1.2	1.7	1.8	1.8	2.1	**2.2**
NANDHYAR VATAM (Methanonic extract)	Copper	1.6	2.1	2.7	-	-	-	1.1	1.6	**2**
	Zinc	**1.3**	**1.4**	**1.8**	**-**	**-**	**-**	**2**	**2.3**	**2.5**

Table.5 Zone of inhibition (72 hrs) against *Fusarium oxysporum* cubense by the Distilled Water extract (50, 100 and 150 µl) of various plant leaves (*Ocimum tenuiflorum*, *Tabernaemontana divaricata*). 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**		
		50	**100**	**150**	**50**	**100**	**150**	**50**	**100**	**150**
TULSI (Distilled water)	Copper	1.4	2	2.5	-	-	-	2	2.8	**3**
	Zinc	1.3	1.5	1.8	-	-	-	-	1.5	**1.7**
NANDHYAR VATAM (Distilled water)	Copper	1.4	2	2.5	-	-	-	1.7	2.4	**2.6**
	Zinc	1.3	1.5	1.8	-	-	-	-	1.4	**1.5**
TULSI (Proponic extract)	Copper	1.4	2	2.5	-	1.6	2.8	1.6	1.5	**2.2**
	Zinc	1.3	1.5	1.8	-	1.6	2.8	1.6	1.9	**2**
NANDHYAR VATAM (Proponic extract)	Copper	1.4	2	2.5	1.6	2.2	2.7	1.5	1.4	**1.9**
	Zinc	1.3	1.5	1.8	1.6	2.2	2.7	1.8	2	**2.1**
TULSI (Hexonic extract)	Copper	1.4	2	2.5	1.4	1.5	1.5	2.2	2.3	**2.5**
	Zinc	1.3	1.5	1.8	1.4	1.5	1.5	-	1.6	**2.2**
NANDHYAR VATAM (Hexonic extract)	Copper	1.4	2	2.5	-	-	-	1.4	1.8	**2.1**
	Zinc	1.3	1.5	1.8	-	-	-	-	1.5	**1.9**
TULSI (Acetonic extract)	Copper	1.4	2	2.5	1.3	-	1.5	-	1.6	**1.5**
	Zinc	1.3	1.5	1.8	1.3	-	1.5	-	1.3	**1.7**
NANDHYAR VATAM (Acetonic extract)	Copper	1.4	2	2.5	-	1.5	1.9	2.1	2.2	**3**
	Zinc	1.3	1.5	1.8	-	1.5	1.9	1.5	2.1	**2.2**
TULSI (Methanonic extract)	Copper	1.4	2	2.5	-	-	-	-	1.9	**2**
	Zinc	1.3	1.5	1.8	-	-	-	1.4	2.1	**2**
NANDHYAR VATAM (Methanonic extract)	Copper	1.4	2	2.5	-	-	-	1.4	1.6	**2**
	Zinc	**1.3**	**1.5**	**1.8**	**-**	**-**	**-**	**2**	**2**	**2.3**

Table.6 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.7 UV Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Distilled water extract of Tulsi (*Ocimum tenuiflorum*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.823**	**0.124**	**0.432**	**0.133**	**0.372**	**0.130**
1 hr	**0.841**	**0.136**	**0.451**	**0.154**	**0.387**	**0.148**
1 ½ hr	**0.864**	**0.152**	**0.476**	**0.176**	**0.411**	**0.162**
2 hr	**0.886**	**0.142**	**0.493**	**0.192**	**0.429**	**0.157**
2 ½ hr	**0.896**	**0.139**	**0.504**	**0.180**	**0.446**	**0.152**

Table.8 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Distilled water extract of Nandhyar Vattam (*Tabernaemontana divaricata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.726**	**0.094**	**0.384**	**0.084**	**0.335**	**0.072**
1 hr	**0.741**	**0.106**	**0.395**	**0.101**	**0.371**	**0.095**
1 ½ hr	**0.768**	**0.137**	**0.423**	**0.134**	**0.393**	**0.121**
2 hr	**0.777**	**0.129**	**0.446**	**0.152**	**0.437**	**0.148**
2 ½ hr	**0.792**	**0.123**	**0.471**	**0.147**	**0.450**	**0.137**

Table.9 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Propane extract of Tulsi (*Ocimum tenuiflorum*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.749**	**0.964**	**0.432**	**0.753**	**0.372**	**0.654**
1 hr	**0.773**	**0.991**	**0.457**	**0.843**	**0.414**	**0.851**
1 ½ hr	**0.798**	**1.057**	**0.483**	**0.900**	**0.437**	**0.893**
2 hr	**0.826**	**1.143**	**0.504**	**1.00**	**0.451**	**0.927**
2 ½ hr	**0.853**	**1.192**	**0.541**	**1.021**	**0.494**	**0.882**

Table.10 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Propane extract of Nandhyar Vattam (*Tabernaemontana divaricata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.724**	**0.247**	**0.384**	**0.153**	**0.334**	**0.220**
1 hr	**0.746**	**0.304**	**0.415**	**0.171**	**0.368**	**0.261**
1 ½ hr	**0.804**	**0.318**	**0.438**	**0.177**	**0.520**	**0.297**
2 hr	**0.846**	**0.322**	**0.457**	**0.182**	**0.547**	**0.289**
2 ½ hr	**0.899**	**0.305**	**0.600**	**0.179**	**0.581**	**0.277**

Table. 1 1 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Hexane extract of Tulsi (*Ocimum tenuiflorum*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.032**	**0.422**	**0.014**	**0.362**	**0.009**	**0.415**
1 hr	**0.074**	**0.471**	**0.021**	**0.379**	**0.015**	**0.426**
1 ½ hr	**0.092**	**0.456**	**0.029**	**0.412**	**0.035**	**0.448**
2 hr	**0.102**	**0.450**	**0.046**	**0.423**	**0.047**	**0.452**
2 ½ hr	**0.110**	**0.463**	**0.067**	**0.430**	**0.052**	**0.459**

Table. 1 2 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Hexane extract of Nandhyar Vattam (*Tabernaemontana divaricata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.058**	**0.057**	**0.014**	**0.063**	**0.012**	**0.072**
1 hr	**0.062**	**0.094**	**0.037**	**0.094**	**0.034**	**0.094**
1 ½ hr	**0.094**	**0.105**	**0.047**	**0.123**	**0.058**	**0.116**
2 hr	**0.109**	**0.119**	**0.061**	**0.144**	**0.072**	**0.137**
2 ½ hr	**0.118**	**0.211**	**0.076**	**0.162**	**0.090**	**0.151**

Table. 1 3 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Acetone extract of Tulsi (*Ocimum tenuiflorum*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**1.156**	**0.684**	**1.167**	**0.711**	**1.166**	**0.696**
1 hr	**1.170**	**0.712**	**1.184**	**0.734**	**1.194**	**0.722**
1 ½ hr	**1.194**	**0.748**	**1.187**	**0.761**	**2.007**	**0.753**
2 hr	**2.002**	**0.792**	**1.194**	**0.799**	**2.016**	**0.781**
2 ½ hr	**2.019**	**0.802**	**2.065**	**0.808**	**2.021**	**0.791**

Table. 1 4 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Acetone extract of Nandhyar Vattam (*Tabernaemontana divaricata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.756**	**0.881**	**0.846**	**0.893**	**0.948**	**0.852**
1 hr	**0.794**	**0.893**	**0.867**	**0.906**	**0.961**	**0.891**
1 ½ hr	**0.901**	**0.927**	**0.924**	**0.927**	**0.994**	**0.915**
2 hr	**0.967**	**0.948**	**0.973**	**0.935**	**1.007**	**0.934**
2 ½ hr	**0.991**	**0.952**	**0.999**	**0.958**	**1.019**	**0.947**

Table. 1 5 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Methanol extract of Tulsi (*Ocimum tenuiflorum*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.861**	**0.904**	**0.902**	**0.911**	**0.951**	**0.872**
1 hr	**0.883**	**0.934**	**0.938**	**0.919**	**0.973**	**0.884**
1 ½ hr	**0.904**	**0.941**	**0.955**	**0.957**	**0.994**	**0.891**
2 hr	**0.937**	**0.935**	**0.969**	**0.928**	**1.112**	**0.916**
2 ½ hr	**0.965**	**0.922**	**0.981**	**0.927**	**1.135**	**0.954**

Table. 1 6 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Methanol extract of Nandhyar Vattam (*Tabernaemontana divaricata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.758**	**0.743**	**0.810**	**0.728**	**0.824**	**0.783**
1 hr	**0.786**	**0.768**	**0.859**	**0.744**	**0.856**	**0.791**
1 ½ hr	**0.817**	**0.791**	**0.921**	**0.761**	**0.887**	**0.821**
2 hr	**0.826**	**0.817**	**0.947**	**0.779**	**0.955**	**0.856**
2 ½ hr	**0.878**	**0.834**	**1.011**	**0.821**	**1.025**	**0.840**

Table.17 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Fresh extract of Tulsi (*Ocimum tenuiflorum*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.524**	**0.023**	**0.482**	**0.038**	**0.537**	**0.040**
1 hr	**0.581**	**0.049**	**0.497**	**0.049**	**0.551**	**0.049**
1 ½ hr	**0.634**	**0.057**	**0.504**	**0.057**	**0.586**	**0.061**
2 hr	**0.672**	**0.073**	**0.524**	**0.042**	**0.624**	**0.067**
2 ½ hr	**0.709**	**0.077**	**0.533**	**0.050**	**0.647**	**0.058**

Table.18 Description of UV absorption spectrum of Copper and Zinc nanoparticles formed from Fresh extract of Nandhyar Vattam (*Tabernaemontana divaricata*) leaves during different time of incubation.

	Copper	Zinc	Copper	Zinc	Copper	Zinc
Time	**435nm**	**385nm**	**560nm**	**435nm**	**680nm**	**560nm**
½ hr	**0.098**	**0.007**	**0.084**	**0.008**	**0.092**	**0.012**
1 hr	**0.123**	**0.016**	**0.096**	**0.017**	**0.099**	**0.024**
1 ½ hr	**0.157**	**0.028**	**0.117**	**0.021**	**0.114**	**0.036**
2 hr	**0.173**	**0.036**	**0.126**	**0.028**	**0.118**	**0.042**
2 ½ hr	**0.177**	**0.039**	**0.134**	**0.034**	**0.125**	**0.046**

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

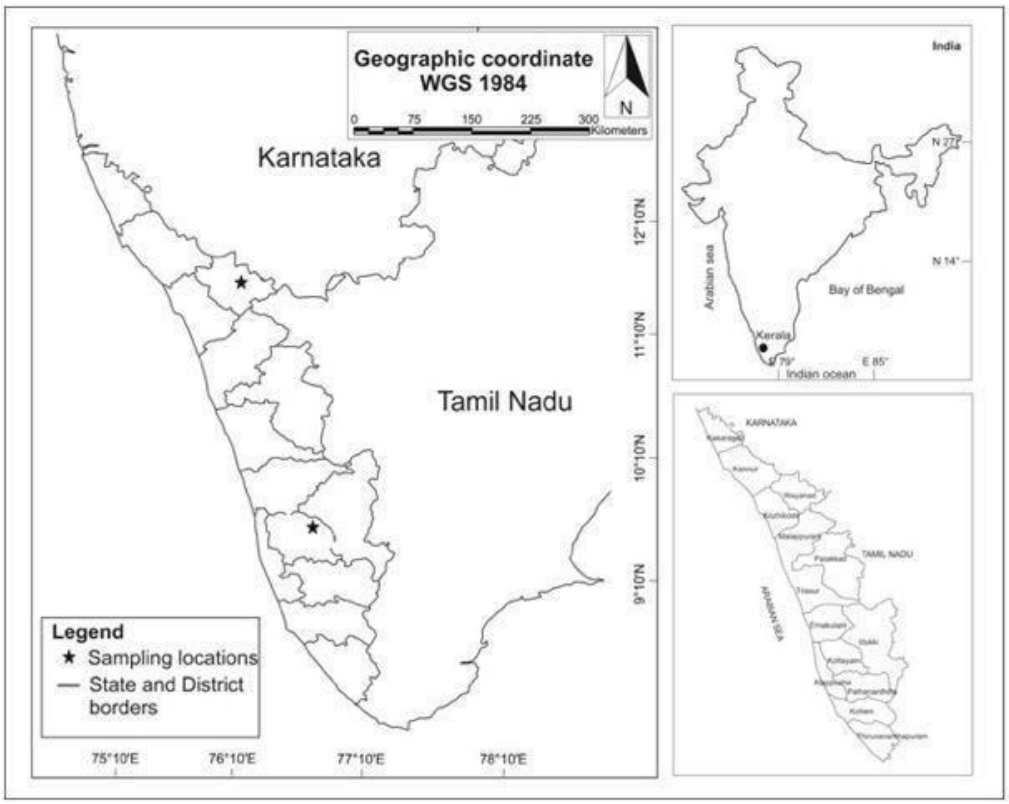


Fig.2 Tulsi (*Ocimum tenuiflorum*) description a) and d) plant with leaves and flowers, b) flowers, c) mature flowers and seeds, e) leaves. Photo courtesy: Wikipedia.

Fig.3 Description of Nandhyar vattam (*Tabernaemontana divaricata*) Description of a) plant from garden, b) plant with leaves flowers and developing buds, c) flower, d) flower cluster, e) and f) plant with flowers and leaves. Photo courtesy: Wikipedia.

Fig.4 Green synthesised Copper nanoparticles *Ocimum tenuiflorum* and *Tabernaemontana divaricata* 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.

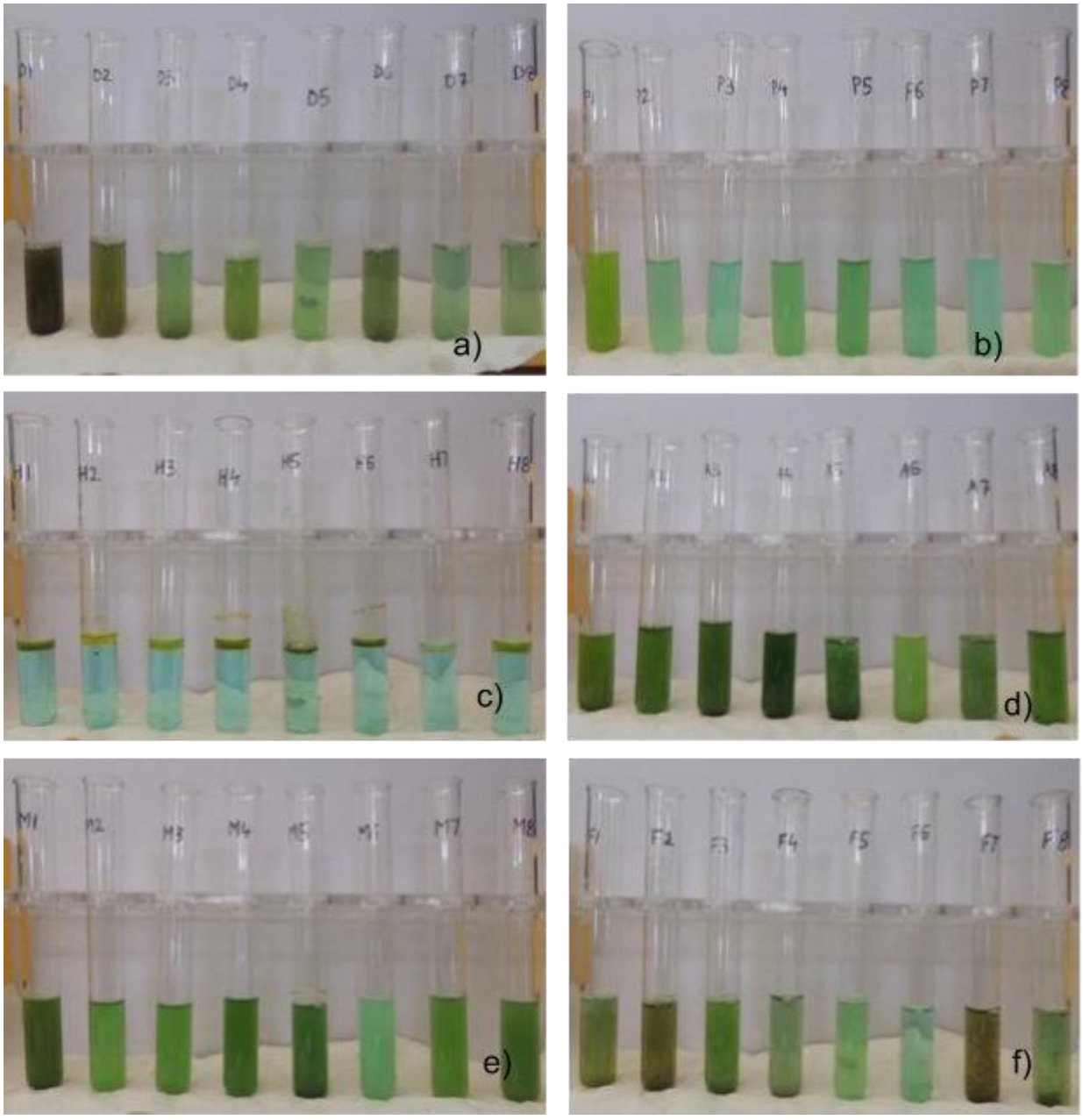


Fig.5 Green synthesised Zinc nanoparticles of *Ocimum tenuiflorum* and *Tabernaemontana divaricata* a) dry leaf distilled water extracts, b) dry leaf propane extracts, c) dry leaf hexane extracts, d) dry leaf acetone extracts, e) dry leaf methanol extracts, f) fresh leaf distilled water extracts.

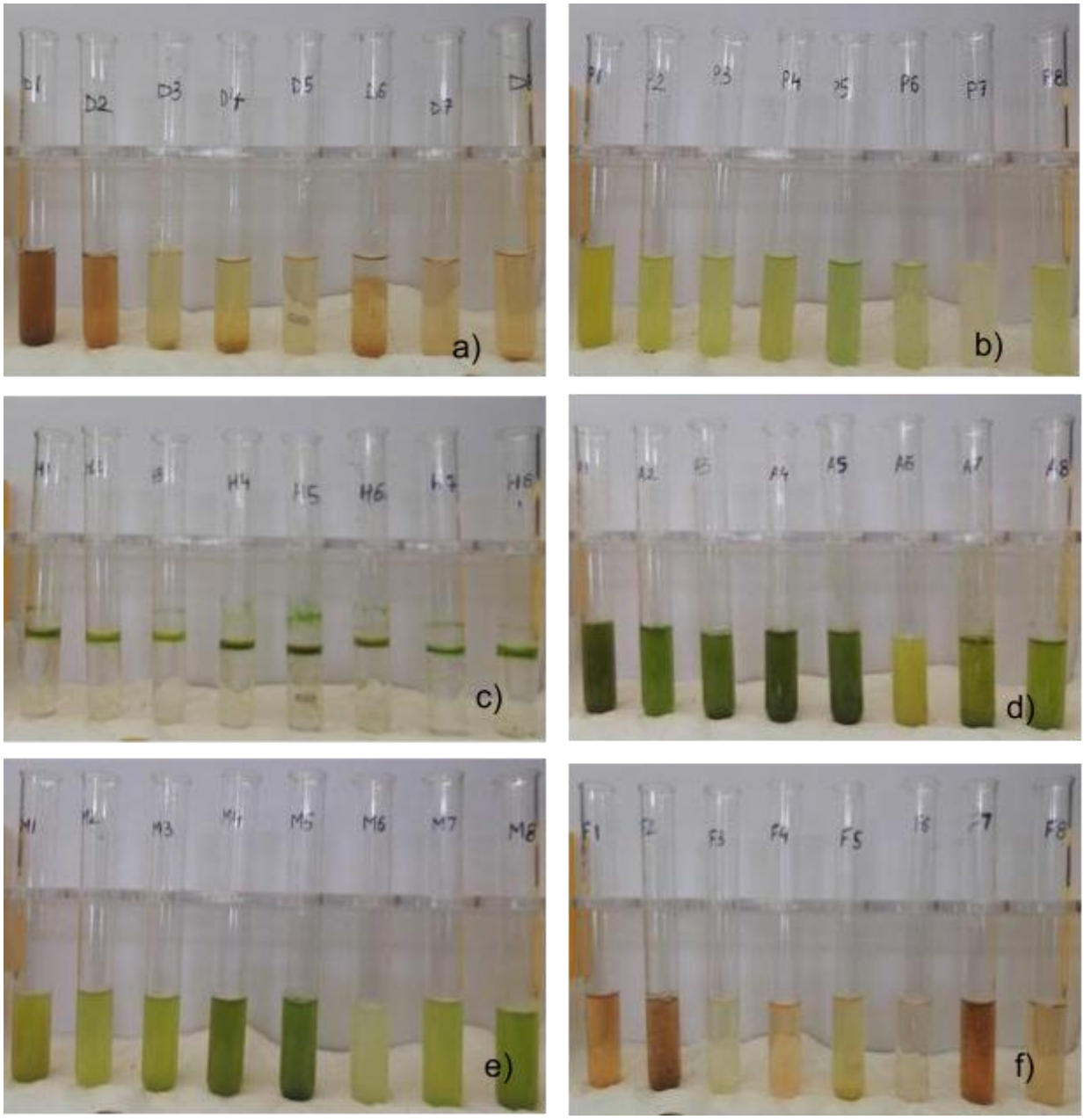


Fig.6 Antifungal activity study using well diffusion method of Tulsi (*Ocimum tenuiflorum*) 24 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test 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).

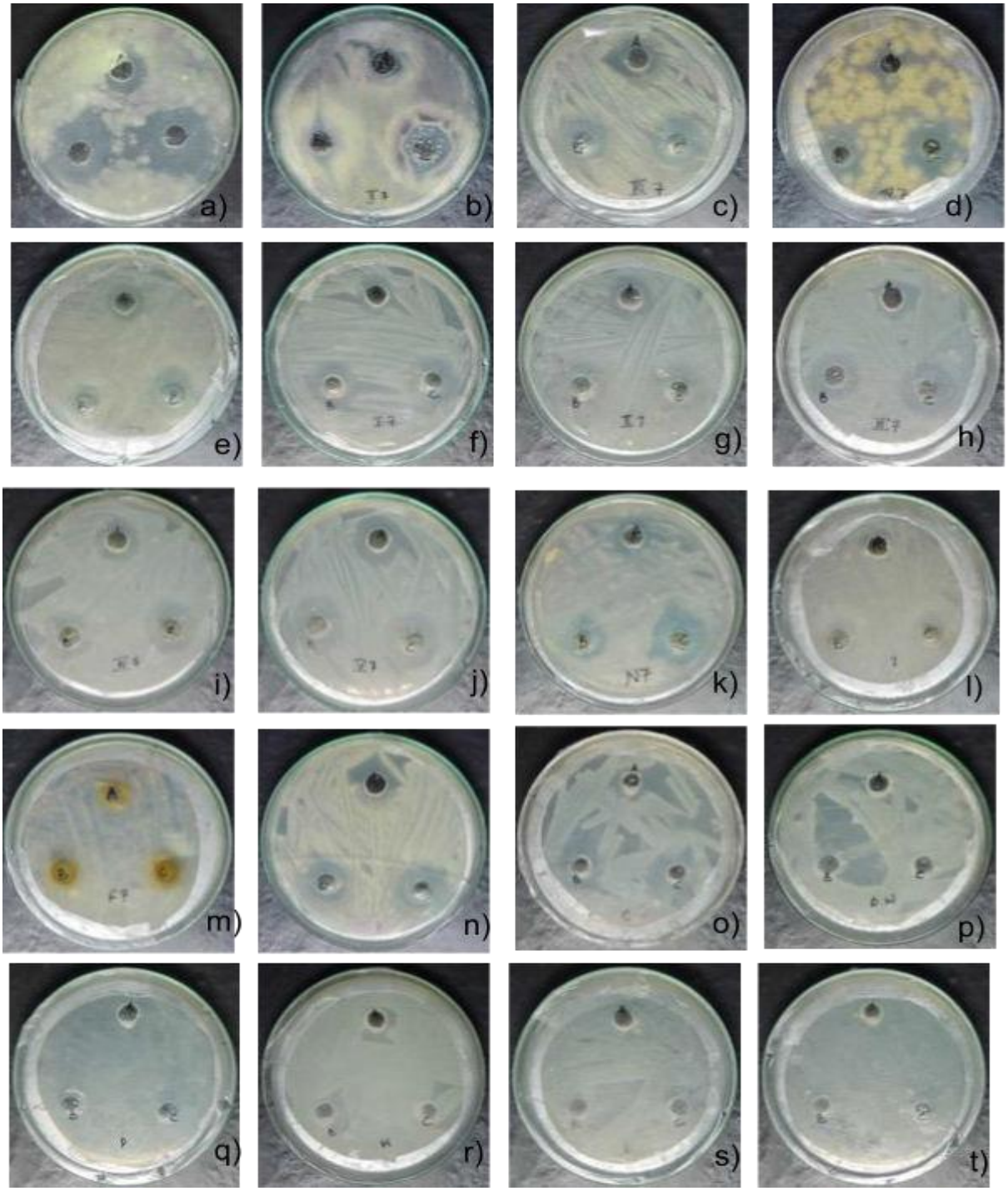


Fig.7 Antifungal activity study using well diffusion method of Tulsi (*Ocimum tenuiflorum*) 48 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test 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).

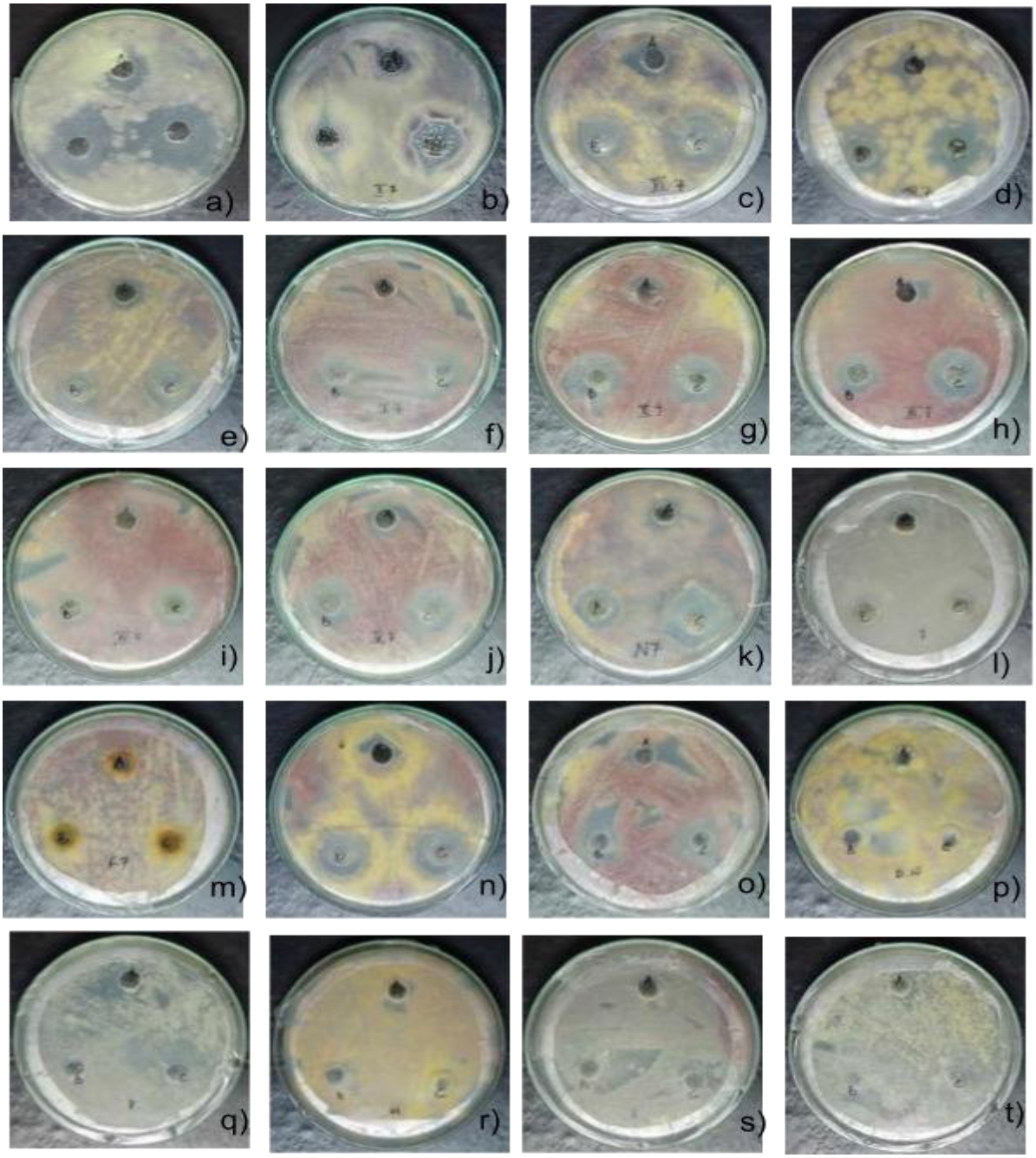


Fig.8 Antifungal activity study using well diffusion method of Tulsi (*Ocimum tenuiflorum*) 72 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned test 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).

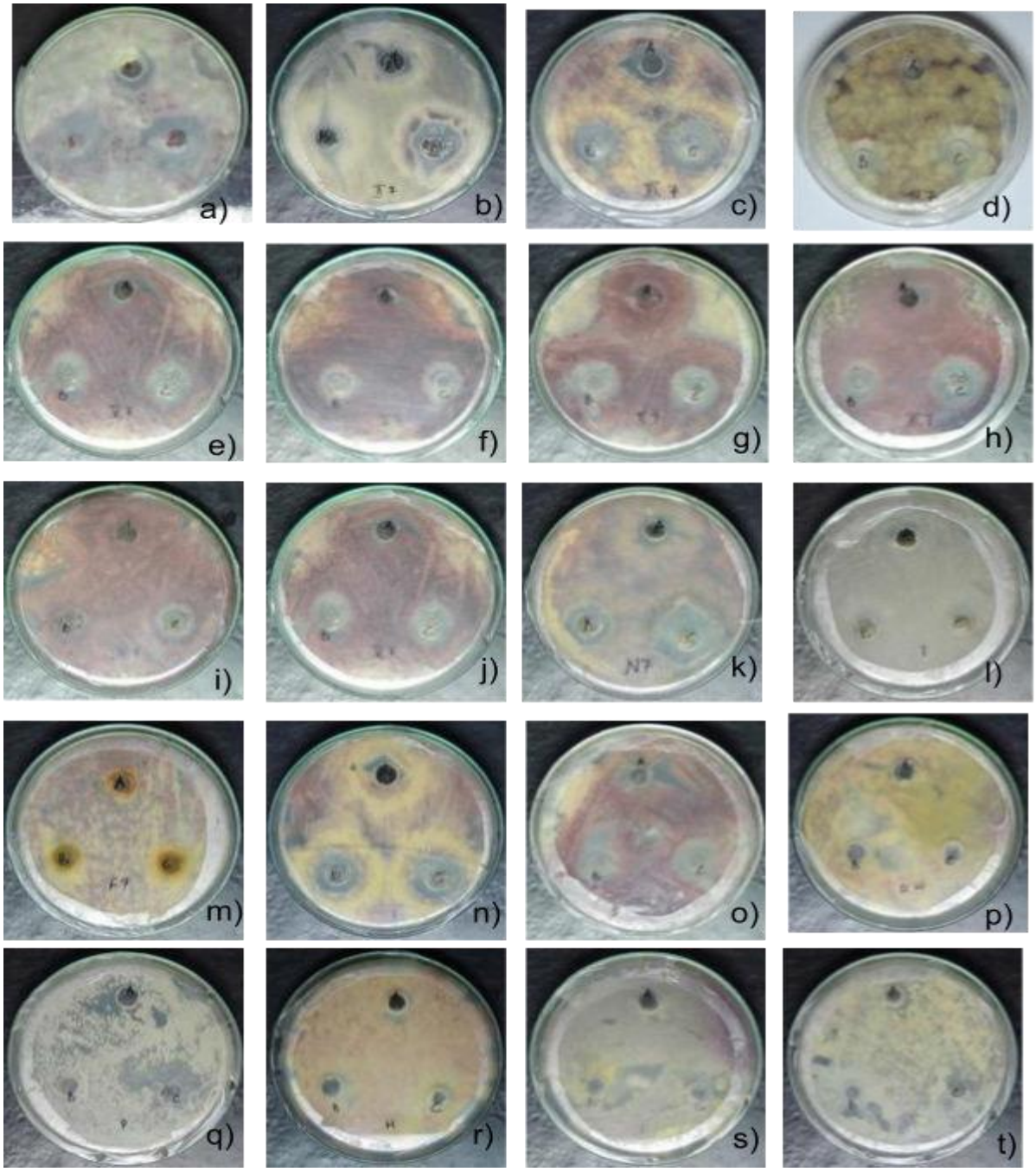


Fig.9 Antifungal activity study using well diffusion method of Nandiyarvattom (*Tabernaemontana divaricata*) 24 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) 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).

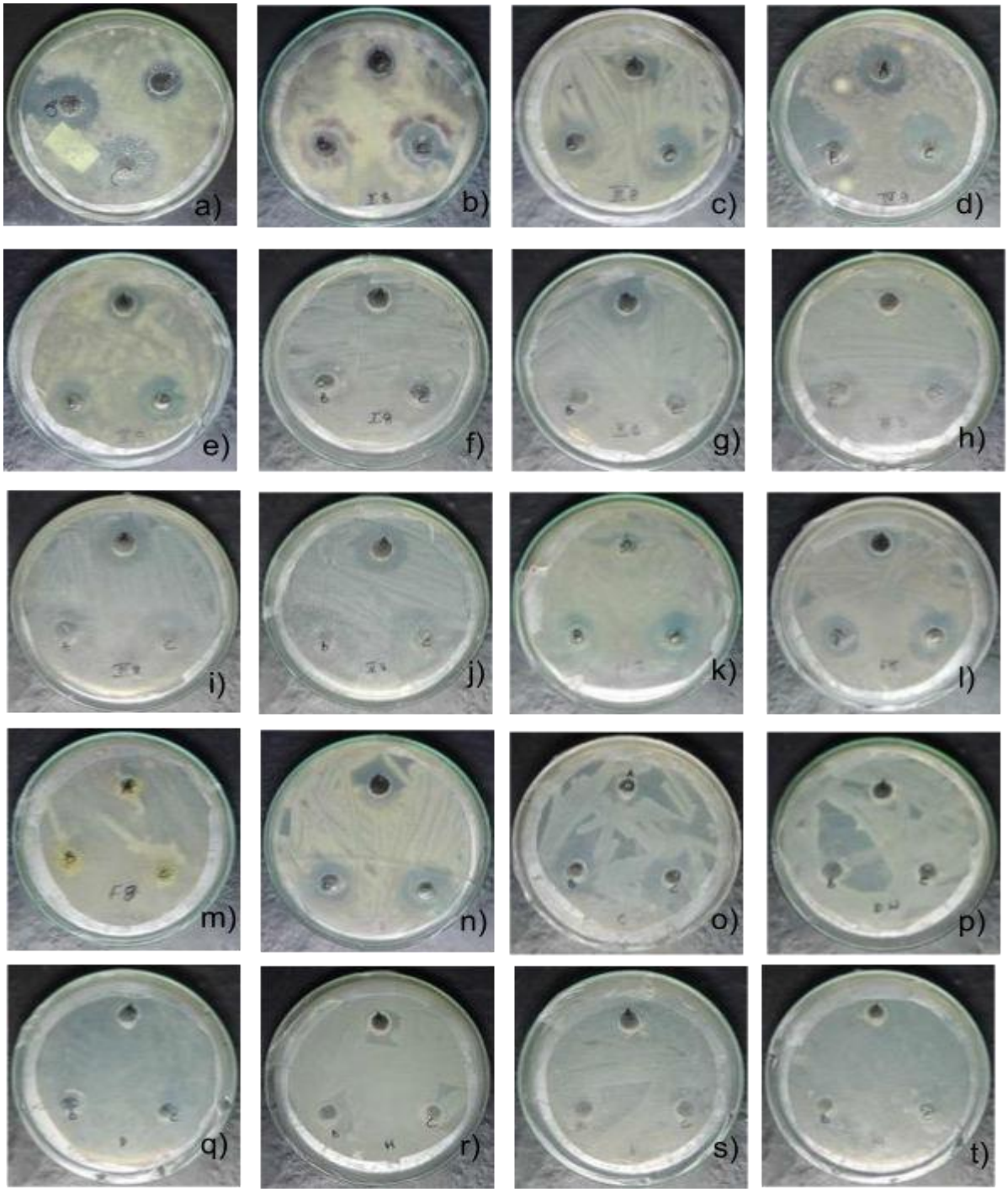


Fig.10 Antifungal activity study using well diffusion method of Nandiyarvattom (*Tabernaemontana divaricata*) 48 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) 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).

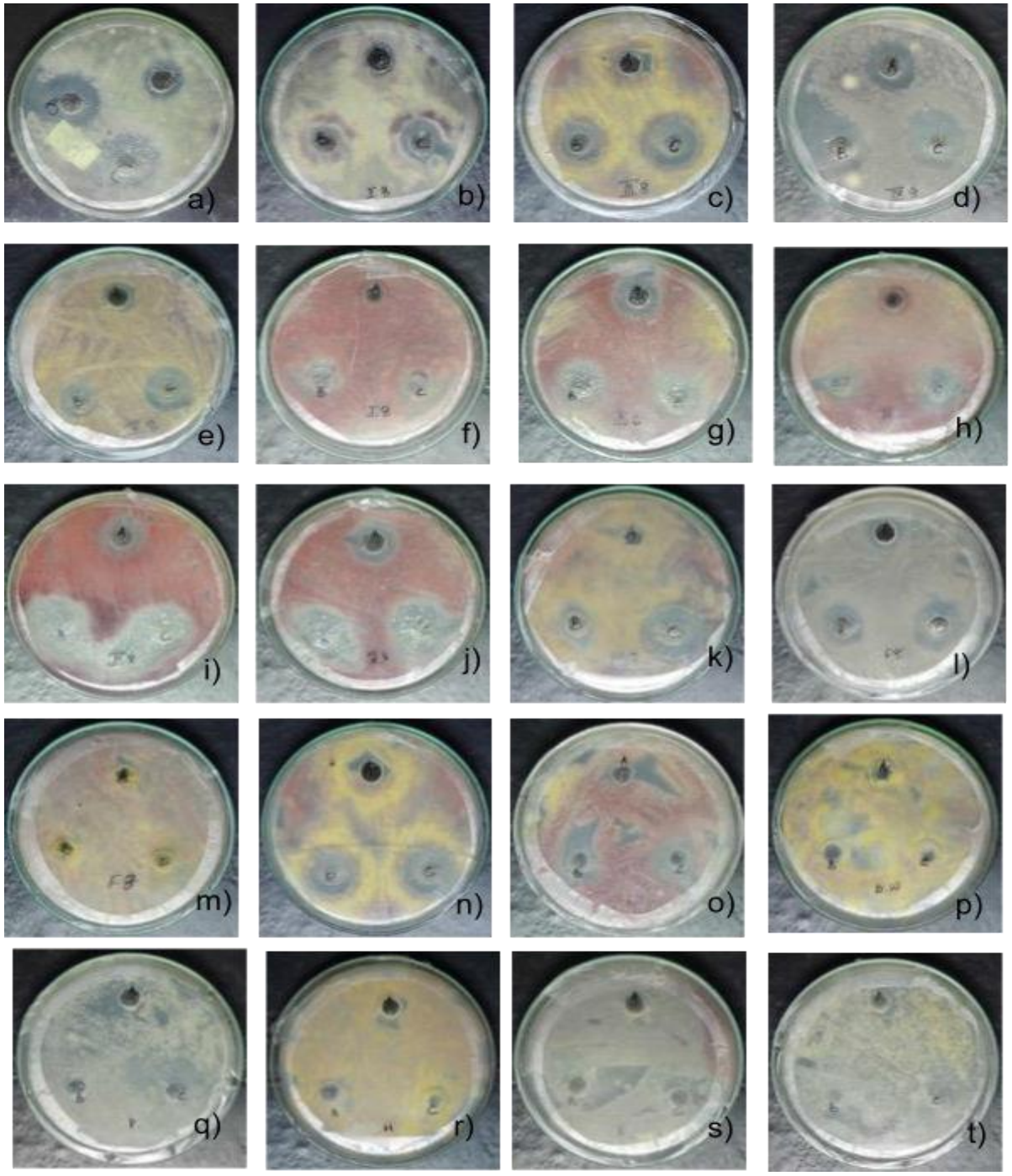


Fig.11 Antifungal activity study using well diffusion method of Nandiyarvattom (*Tabernaemontana divaricata*) 72 hrs leaf extract nanoparticles (Cu) and (Zn) a) green synthesised Cu nanoparticle test plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) 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).

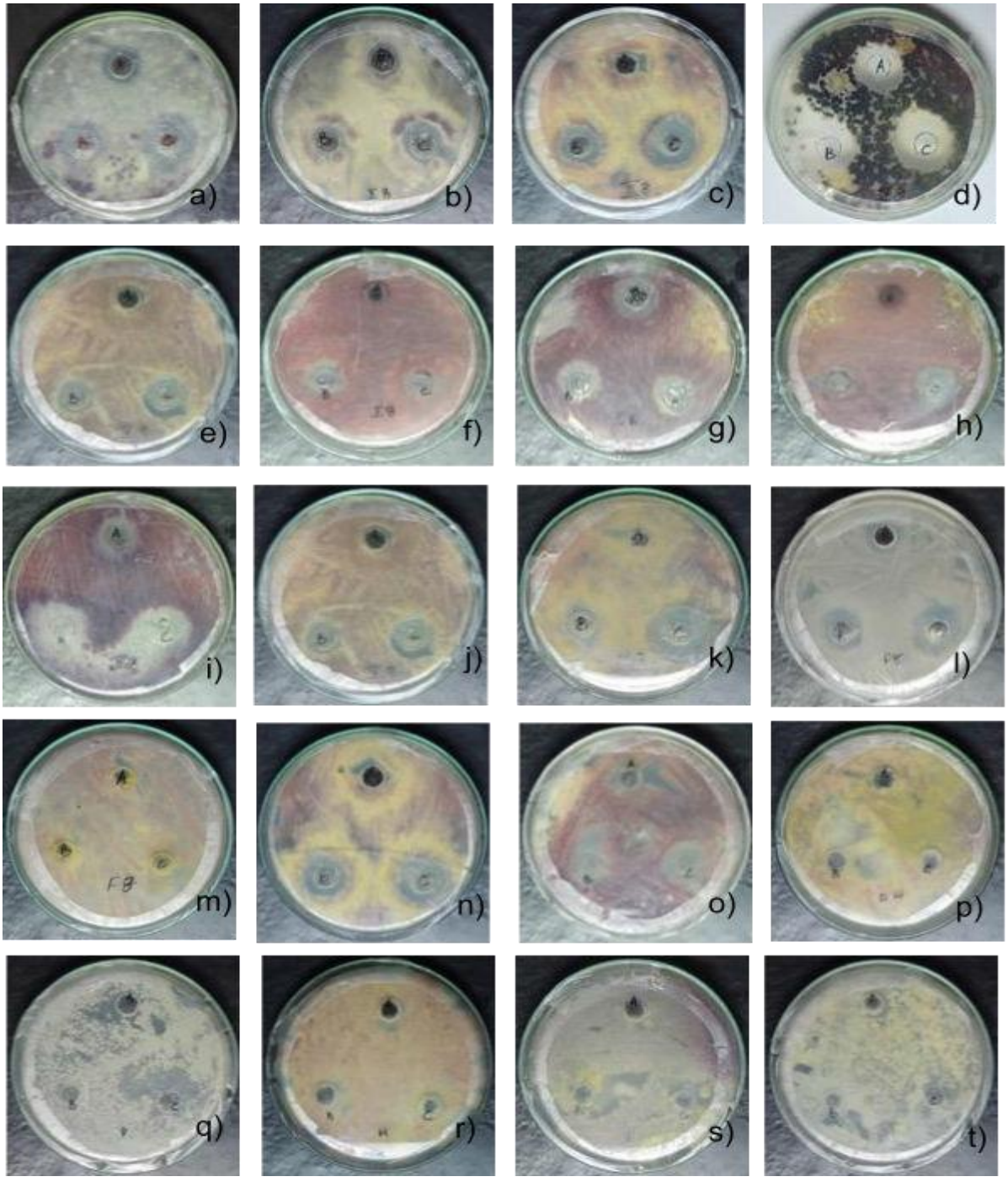


Fig.12 Antifungal activity study using well diffusion method of Tulsi, (*Ocimum tenuiflorum*) 24 hrs leaf dry extract a) control plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned control plate in same order for Nandiyarvattam (*Tabernaemontana divaricata*), k) to o) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

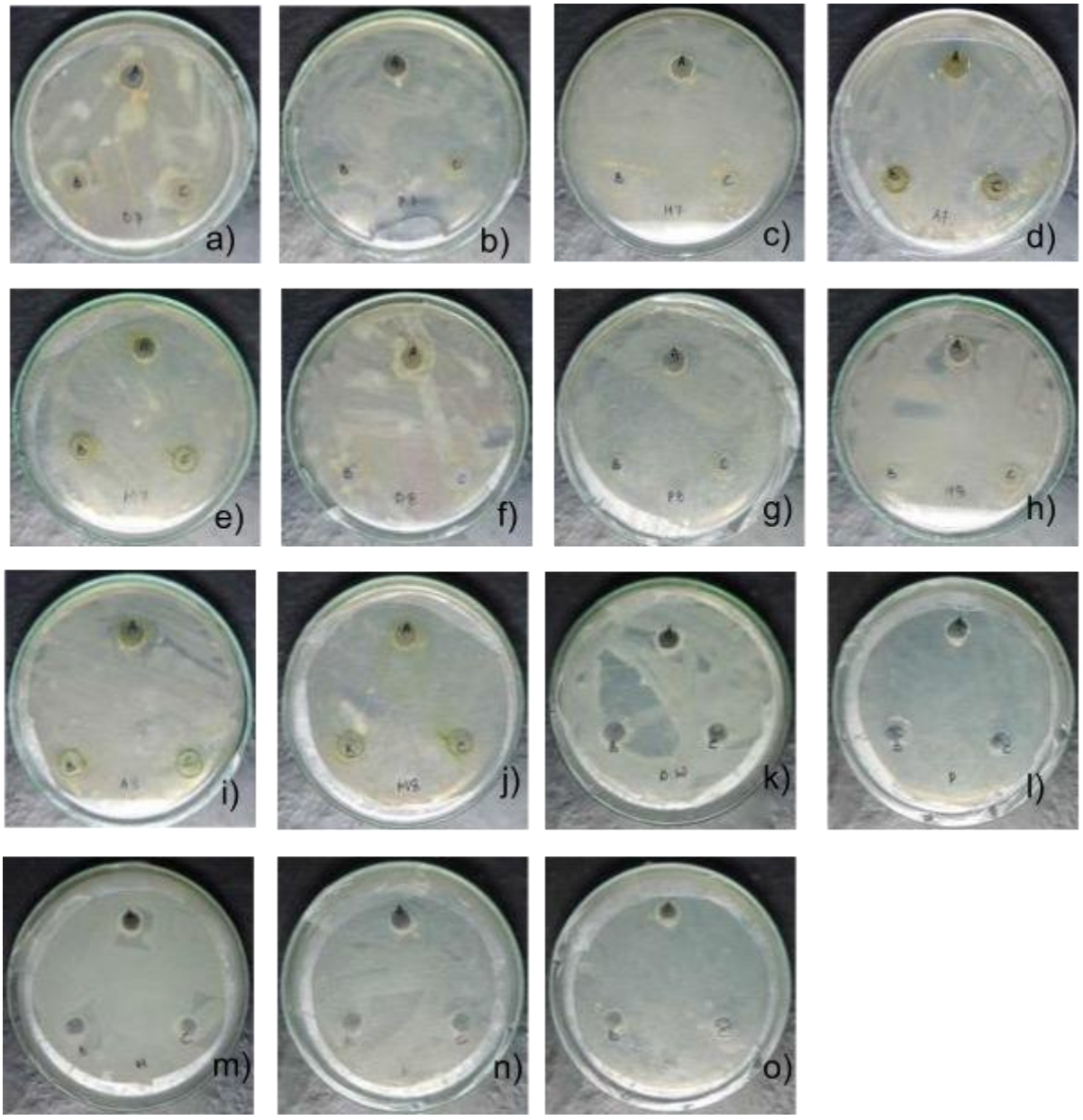


Fig.13 Antifungal activity study using well diffusion method of Tulsi, (*Ocimum tenuiflorum*) 48 hrs leaf dry extract a) control plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned control plate in same order for Nandiyarvattam (*Tabernaemontana divaricata*), k) to o) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

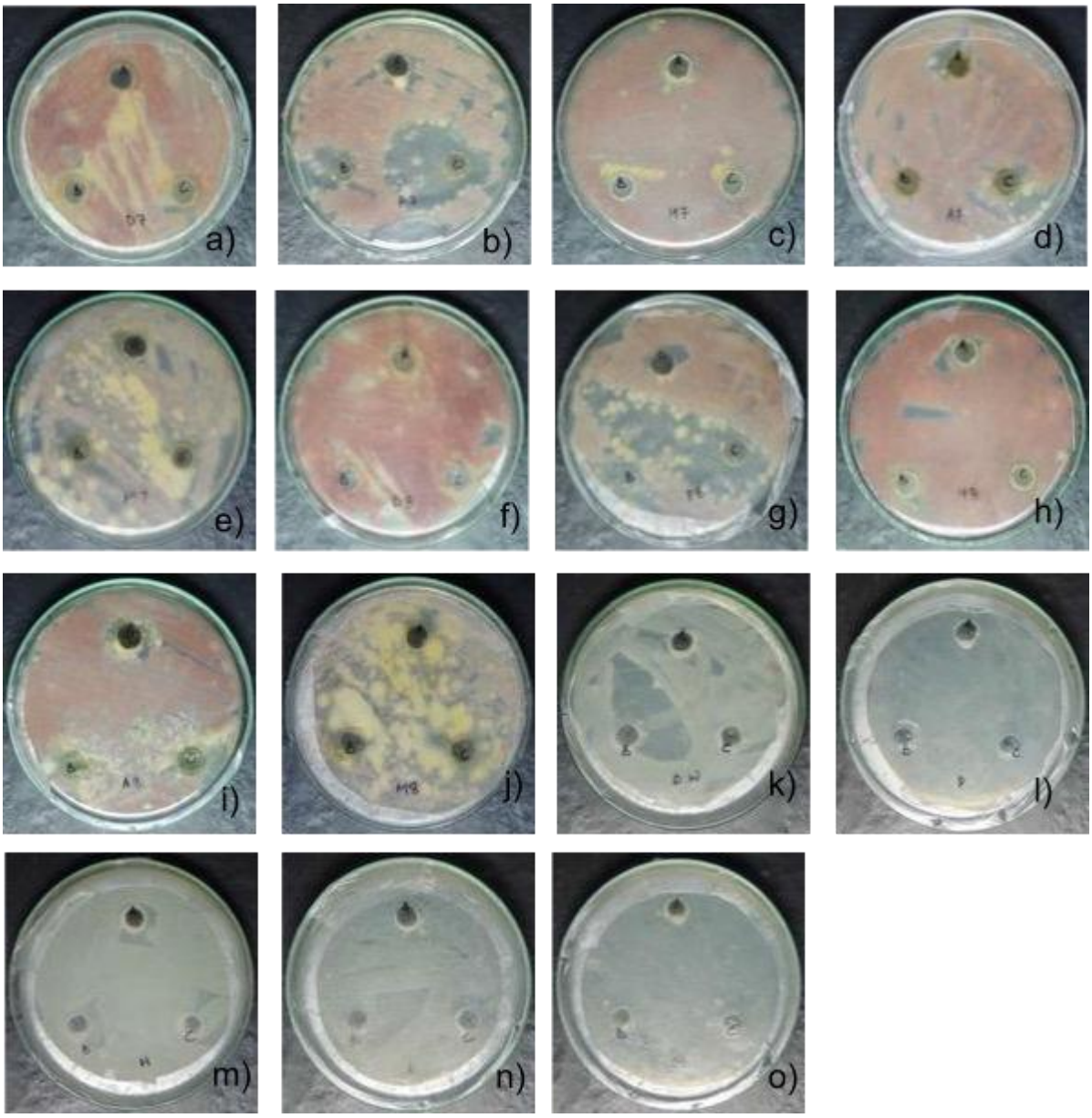


Fig.14 Antifungal activity study using well diffusion method of Tulsi, (*Ocimum tenuiflorum*) 72 hrs leaf dry extract a) control plate (Distilled water; 50, 100 and 150 µl), b) Propane, c) Hexane, d) Acetone, e) Methanol, f) to j) above mentioned control plate in same order for Nandiyarvattam (*Tabernaemontana divaricata*), k) to o) solvent control plates (Distilled water, Propane, Hexane, Acetone, Methanol).

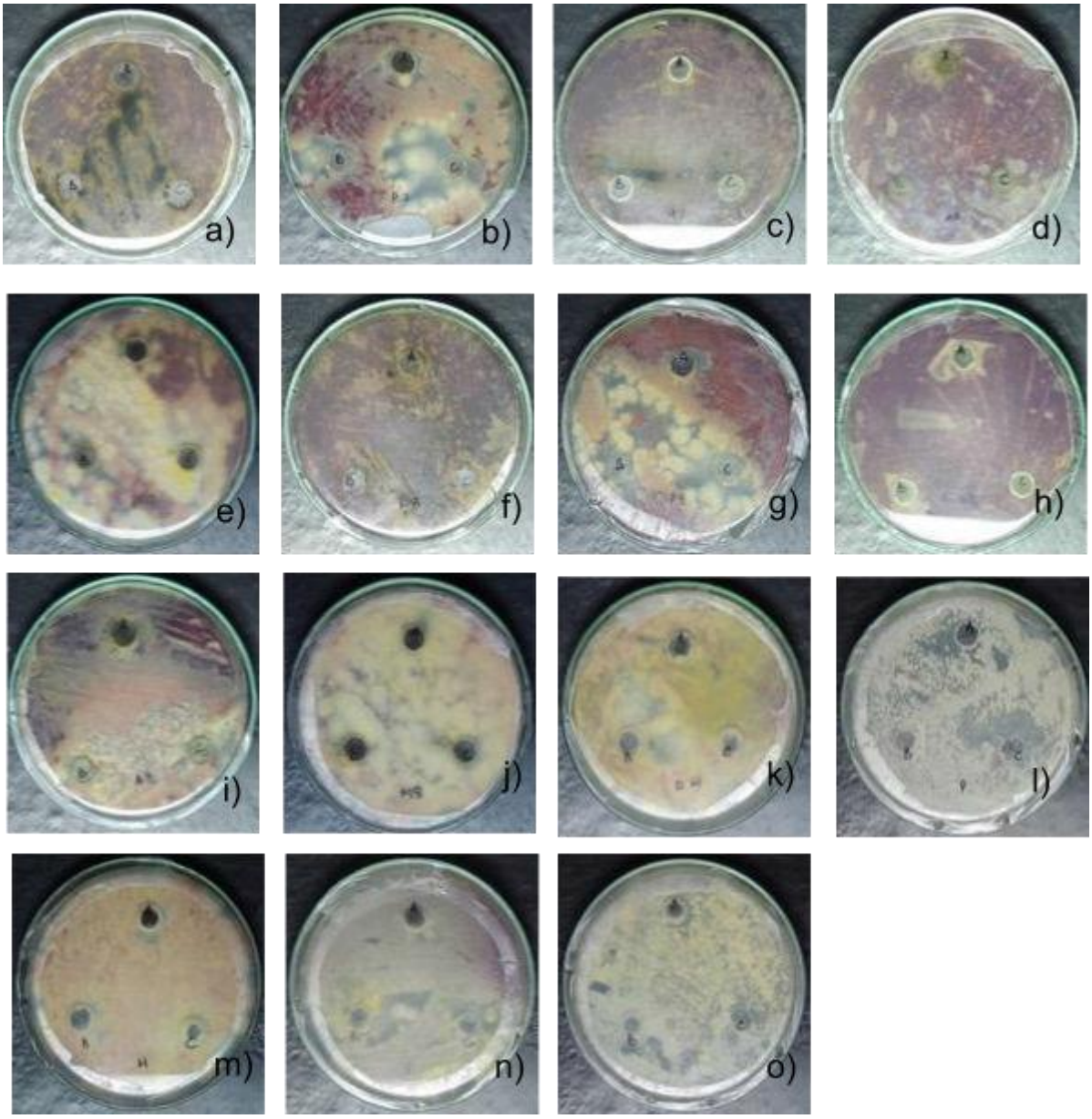


Fig.15 Copper nanoparticle formation of Tulsi (*Ocimum tenuiflorum*) dry leaf extract under SEM imaging system with various resolutions.

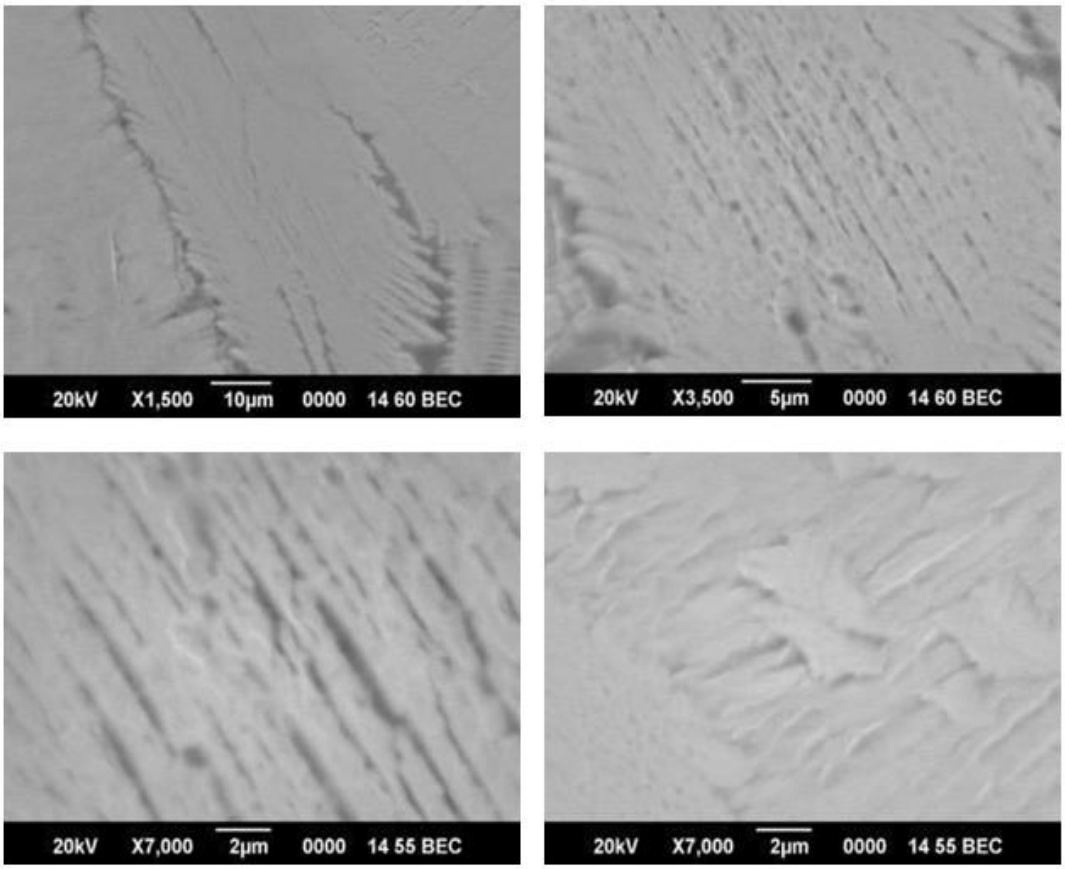
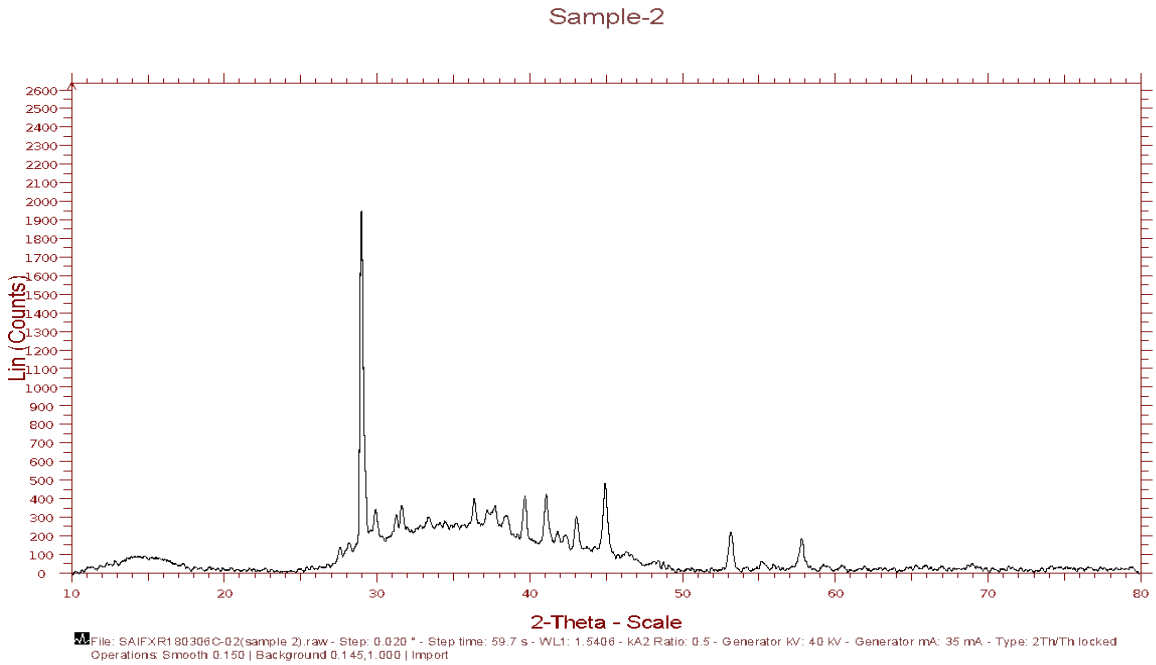
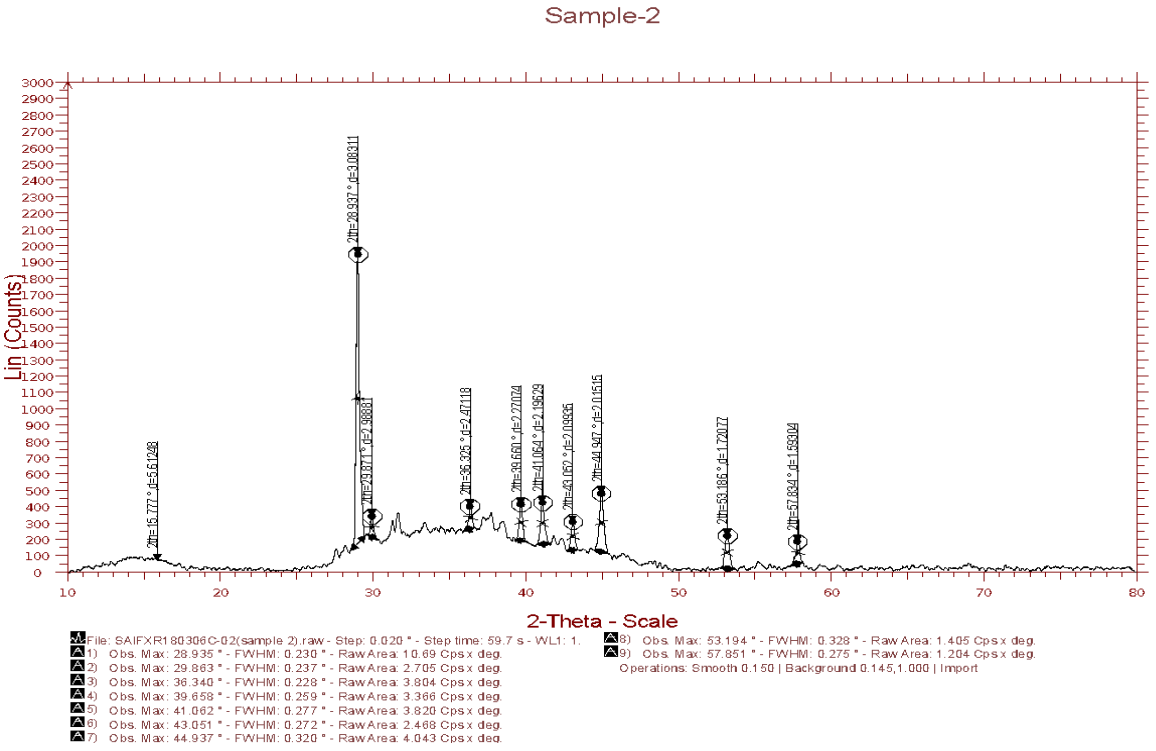


Fig.16 Copper nanoparticle formation of Tulsi (*Ocimum tenuiflorum*) dry leaf distilled water extract under XRD imaging system.





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).

Results and Discussion

Synthesis of nanoparticles

Nanoparticles were synthesized from the leaf extracts of *Ocimum tenuiflorum* and *Tabernaemontana* divaricata using solvents like distilled water, propane, hexane, methanol and acetone.

Copper nanoparticles

Copper nanoparticles were synthesized from leaf extract of different plants (*Ocimum tenuiflorum* and *Tabernaemontana divaricata*).

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

Zinc nanoparticles

Zinc nanoparticles were synthesized from leaf extract of different plants (*Ocimum tenuiflorum* and *Tabernaemontana divaricata*).

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.

Characterization of nanoparticles

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 *Ocimum tenuiflorum* and *Tabernaemontana divaricata* 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.

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 Ocimum tenuiflorum* and *Tabernaemontana divaricata* with each solvent.

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

Antifungal assay

The leaf extracts of *Ocimum tenuiflorum* and *Tabernaemontana divaricata* with solvents distilled water, propane, hexane, methanol and acetone and the fresh extract showed the growth inhibitory effects against *Fusarium oxysporum cubense*.

Tulsi (*Ocimum tenuiflorum*)

One of the best results have been observed with both nanoparticles for more than 3 days and showed the highest value as 3.3cm with the water solvent forming copper nanoparticles. Methane and acetone extracts also had better zone of inhibition with zinc nanoparticles when compared with other plants.

Nandhyar vattam (*Tabernaemontana divaricata*)

A similar result was obtained with propane and acetone extracts with Cu and Zn nanoparticles with the least inhibitory action shown by the hexane extract. The results shows that leaf extracts of, *Ocimum tenuiflorum* and *Tabernaemontana divaricata* 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 Distilled water, Methanol and Propane as a good solvents and Tulsi as one of the best remedy against the Panama wilt disease.

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