# FORMULATION OF POLYHERBAL ENRICHED SEAWEED WITH ANTI-PLANT PATHOGEN PROPERTY

**A PROJECT REPORT**

***Submitted by***

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## CERTIFICATE

This is to certify that **ASWINI P.R (111416214701)** & **NANDHINI.M (111416214017)** currently pursuing final year **B.Tech, Biotechnology, PRATHYUSHA ENGINEERING COLLEGE** have successfully completed the project entitled **" FORMULATION OF POLYHERBAL ENRICHED SEAWEED WITH ANIT-PLAT PATHOGEN PROPERTY** from **08/12/2019** to **19/03/2020** at **Biozone Research Technologies Private Limited, Chennai-600 017**.

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# DECLARATION

I hereby declare that the project report titled "**Formulation of polyherbal enriched seaweed with antiplant pathogen property**" submitted to the Department of Biotechnology, Prathyusha Engineering College - Thiruvallur, Chennai,inpartialfulfillmentoftheawardofthedegreeBachelorofTechnology in Biotechnology, is the record of the original work carried by me under the guidance of **Dr.Aneesh Nair, Biozone Research Institute** during the period of december 2019 to March 2020. I further declare that the results of the work have not been previously submitted for the award of any degree ordiploma.

Place:Thiruvallur **ASWINI.P.R**

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Now a days demand for the organic foods increased the demand of the biofertilizer production .The present study is to isolate seaweed biofertilizer &formulate of polyherbal enriched seaweed biofertilizer .The study is also focused to study the germination and growth parameters of biofertilizers treated maize plant .The plant with antifungal activity *Acalypha indicia* ,*Calotropis gigantean* and *Lawsoina inermis* were collected .Antifungal activity of the plant extracts was analysed by well diffusion method against to the plant fungal pathogen Curvularia lunata .The result revealed that the chloroform extract of *Lawsoina inermis* shows 75%inhibition of the plant fungal pathogen *Curvularia lunata*.Simultaneously ,seaweed liquid fertilizer extracted from the macroalage *Sargassium muticum* . The plant grown in with 10% concentration of seaweed and 10% chloroform extract of *Lawsonia inermis* plant leaf in the condition of seed soaked +fertilizer sprayed is found to be the tallest plant when compared to all the plant grown in different conditions.

**KEYWORDS:** *Acalypha indic*a, *Calotropis gigantea***,** *Lawsonia inermis***,** Antifungal**,** Biofertilizer preparation, Plant growth.

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*A.indica* - Acalypha indica

$^0$C - DegreeCelsius

g - gram

µg -microgram

mg -milligram

ml -millilitre

mm -millimetre

PDA -potatodextroseagar

*C.lunata* - *Curvularia lunata*

*L.inermis* - *Lawsonia inermis*

*C.gigantea* - *Calotropisgigantea*

*s.muticum* – *sargassummuticum*

# AIM AND OBJECTIVE

## AIM:

Our aim is to formulate the polyherbal from three different plants *Acalypha indica* (kuppaimeni), *Lawsonia inermis*(maruthani), C*alotropis gigantea*(erukan) with seaweed (*sargassum muticum*) to produce biofertilizer against to the plant fungal pathogen *Curvularia lunata*.

## Objective:

1. To identify plants with antifungal activity against *Curvularialunata*.

2. Toisolate seaweed biofertilizer and formulate polyherbal enriched seaweed biofertilizer

3To study the germination and growth parameters of biofertilizer treated maizeplants.

# CHAPTER 1

# INTRODUCTION

Nowadays excessive chemical inputs usage, hard soils, low pest resistance and stagnant/ declining yields have become global challenges in agriculture. Majority of farmers in these days are struggling to balance higher input costs, developing their fields and maintenance of yields. So this has led to a subsequent decline in profits with farmers actively searching for sustainable solutions as a business altogether.

Therefore aggressive use of harsh chemical inputs leads to a decline in beneficial soil biology to a greater extent. This leads to higher requirement of chemical fertilizers as a major nutrient source in the absence of adequate soil biological activity. Presence of high salt deposits and loss of organic matter in soil further leads to hardening of soil, which greatly impact the crop yieldsaswellasqualityofthecrops.Thereforefarmersawarenessofsuchissuesareunableto implement sustainable solutions.

Ineffortstosolvesuchproblemsandensureorganicallysafeandhealthyfood,organicfarming is often suggested as an option. However, the reason for its failure to implement it leads to lower yields and losses during the first few seasons of this transition towards organic farming methods. Such losses are not affordable for most farmers from trying such newmethods.

Nowadays new generation technologies are available to solve this sort of problems. These technologies are greatly biologically boosting technologies that promote the growth of beneficial soil biology whilst improving soil quality, water holding capacity and % of organic matter.Thus,theybestowdualbenefitsofbiofertilizersaswellassoilconditioners.Thisallows farmers to benefit from enhanced organically developed high-quality yields along with healthiersoils.

## *1.1 Acalyphaindica*

*Acalypha Indica* (FIG.1.1) known as kuppaimeni in tamil is an annual weed it belongs to the family Euphorbiaca. *Acalypha indica* is an herbaceous annual that has catkin likeinflorescenceswithcup-shapedinvolumessurroundingtheminuteflowers(Schmelzer

and gurib-fakim ,2008). It is a common weed in many parts of Asia. It grows in the common farmlands,gardens,roadsideswastelands.Partsusedareleavesroots,stalkandflowers,ithas been reported to be useful in treating Pneumoniae, asthma, rheumatism and several other ailments ,the dried leaves of was made in to a poultice to treat bedsores and wounds and the juiceofis*AcalyphaIndica*addedtooilarelimeandusedtotreatavarietyofskindisorder ,Leaves possess laxative properties (a substitute for senega) used in the form of powder decoction cures diseases of the teeth and gums, burns ,toxins of plants and mixed origin stomachpain,diseasesduetopitta,bleedingpiles,irritations,stabbingpain,wheezing,sinusitis and neutralizes predominance of the Kabha Factor. The ethanolic extracts of Heliotropium Indicum , Plumbago zeylanicum and *Acalypha indica* were evaluated for their wound healing activityinrates(sureshReddyetal.,2002).Themajorphytochemicalconstituentsarealkaloids acalypus and alcyphine (Kirtikar and Basu,1975). This plant is used as diuretic antihelmintic andforrespiratoryproblemsuchasbronchitis,asthmaandpneumonia(Varier,1996).Theroots of *Acalypha indica* is used as laxative and leaves for scabies and other cutaneous diseases (perry,1980).The plant has many traditional medical uses .In Madagascar ,the crushed plant is usedforskinParasites.InMauritius,theshapeofcrushedleavesmixedwithsaltoradecoction of palnt, is used for seabies and other skin problems. In Madagascar, the crushed plant is used for skin Parasites. In Mauritius, the shape of crushed leaves mixed with salt or a decoction plant, is used for seabies and other skin problems. In the Seyehelles, infusion or decoction is taken for asthma, and also to clean the liver and kidneys. The root decoction is also Taken for intestinalwormsandstomache.Theleavesshapeistakenasanemeticinthecaseofpoisoning. A leaves infusion is also taken sapurgative and vermifuge in Madagascar. In East Africa sap of the leaves is used for eye infections. Leaf powder is used for maggot-in fested wounds *Acalypha indica* is listed in the pharmacopoeia of india as an expectorant to treat the asthma and Pneumonia. This plant is held in high system traditional Tamil Siddha medicine as its belivedtorejuvenatethebody.TheplanthasalsobeeneatenasavegetableinAfricaandIndia. But care needs when eating it since it contains several alkaloids as well as hydrocyanic acid. This plant has been used extensively in herbal medicine in many tropical and subtropical countries(Ramachandran,2008).

**Fig 1.1** ***Acalypha indica***

## *1.2 Lawsoniainermis*

*Lawsoniainermis*syn.*Lawsonia*albaknownashennaisafloweringplant,itistakeplace inthegenus*Lawsonia*(Sıddiqueetal.,2003;Arunetal.,2010).Itisnativeforsomesubtropical andtropicalregionsofAfricaandSouthernAsiainsemi-aridzones.Henna*(Lawsoniainermis*) is cultivated commercially throughout Pakistan, India, Iran, Libya and Sudan for its valuable leaves (Saadabi, 2007). Henna plant is deciduous, has a perennial shrub which is reaching a height of up 2,5 - 5m. The plant leaves are small, lanceolate, dark-green, opposite and have shortpetioles.Theleavesofplantcontainsaredorangecolorcomponent,lawsone(2-hydroxy- 1, 4-Napthoquinone). Lawsone (2-hydroxy-1, 4- Napthoquinone) is easily bonding with protein, and thus it has been used to dye skin, hair and fingernails (Sıddique et al., 2003; RahimanandTaha,2011).Theplanttraditionallyuseforitsredorblackcoloringtohands,feet and hair in some occasions such as weddings etc. (Saadabi, 2007). For the cooling effect of henna, the paste form is used to bring down fever. Henna is beleived as a medicinal plant, because of its antibacterial effects especially on gram positive bacteria, antifungal activity against dermatophytes, wound healing, antitumoral effects, hypotensive, astringent and sedative effects. We have seen it as a folk medicine in using against headache, jaundice and leprosy Several studies are being carried towards it activates like cytotoxic, hypoglycaemic, antimicrobial, antibacterial, antioxidant, trypsin inhibitory, wound Healing, analgesic, anti-corrosin, anti-inflammatory, antiparasitic, tuberculostatic, hepatoprotective, anti-tumoral activity(Berenjietal.,2010;Elmanamaetal.,2011;Karpeetal.,2011;Rayavarapuetal.,

2011).Thepasteformordecoctionofhennaleavesarealsousedasaprophylacticagainstskin inflammation(Sıddiqueetal.,2003).Phytochemicalcharacterisionof,*Lawsoniainermis*Plant produces a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoid Many studies have already been done on the spot disease of paddy plant, but to the best of our knowledge, the similar reports on maize are absent .The effect of the *Lawsonia inermis* extracts on *Curvularia lunata* which is the causative organism of the spot disease in maize.

**Fig 1.2 *Lawsonia inermis***

## *1.3 Calotropisgigantea*

*Calotropis gigantean* genera comprise of two species, with 90% inhabaiting southern Asian country and are most endemic to the India, Indonesia, Malaysia, Thailand and Srilanka, China. *Calotropis gigantea* a weed plant commonly known as giant milk weed. The plant is belonging to Apocynaceae family which *Calotropis gigantean* is a weed plant commonly knownasgiantmilkweed.TheplantisbelongingtoApocynaceaefamilywhichincludeslatex bearing plants. *C.gigantea* is known for various medicinsal properties in traditional medicinal system and use to cure a variety of diseases. In last few decades, *C. gigantea* is extensively studied for its medicinal properties by advanced scientific techniques and a active compounds have been isolated from the different parts of the plant and were analysed pharmacologically. The plant is reported for analgesic activity, antimicrobial activity, antioxidant activity, anti-pyretic activity, insecticidal activity, cytotoxicity activity, hepatoprotectiveactivitypregnancyinterceptiveproperties,purgativeproperties,procoagulant activity and wound healing activity. The medicinal properties of this plant represent it as a valuablesourceofmedicinalcompound.Itisalargeshrubgrowingto4m(13ft)tall.Ithas

clusters of waxy flowers that are either white or lavender in colours. Each flower consists of five pointed petals and a small " crown" rising from the center which holds the stamens. The aestivation found in *Calotropis* is valvate i.e. sepals or petals in a whorl just touch one another at the margin, without overlapping. The plant has oval, light green leaves and milky stem. The latex of *Calotropis gigantea* contains cardiac glycosides, fatty acids, and calcium oxalate. Phyto chemical studies on *Calotropis* have afforded several types of compounds such as Cardenolide, triterpenoids, alkaloids, resins, anthocyanins and proteolytic enzymes in latex, flavonoids, tannins, sterol,saponins, cardiac glycosides. Flowers contain terpenes, multiflorenol, and cyclisadol. *C. gigantea* is also against the antifungal activity of *Curvularia lunata*.

**Fig 1.3 *Calotropis gigantea***

## *1.4 Curvularialunata*

The causal organism of leaf spot of maize *Curvularia lunata* (Wakker) Boedjn is soil and seed borne pathogenic fungus. It is characterized by production of brown geniculate conidiophores with curved conidia in host tissue and culture media.

### 1.4.1 Ecology

*Cochlioboluslunatus* has a wide spread distribution ,though it is especially prevalentinthe tropics and subtropics. Infection is caused by airborne conidia and ascospores, however, sclerotioid *C. lunatus* can also survive in the soil. The optimal temperature for in vitro growth and infection ranges from 24-30 °C while death results from exposure at 59 °C for 1minute duration, or 55 °C for 5minute duration. Successful plant host infection requires the host surface to be wet for 13 hours. The majority of clinical cases have been reported in India, the United States, Brazil, Japan and Australia.

### 1.4.2 Morphological characteristics

*Curvularia lunata* (Wakker) Boedijn Colony of *Curvularia lunata* is brown, gray, or black, cottony, hairy, or cushion-like and spreads loosely. Conidia 3-5 celled with middle cell enlarged, dark and curved. Hyphae *of Curvularia lunata* are branched and septate. Conidiophores are erected unbranched and septate. The size of conidia measured 18- 29×8-10µminsize.Theanamorphofthisfungusisknown*as Curvularialunata*,while *Cochliobolus lunatus* denotes the teleomorph or sexual state. They are, however, the same biological entity. *Cochliobolus lunatus* is the most commonly reported species in clinical cases of reported *Cochliobolus* infection.

### 1.4.3 Plant diseases

*Cochliobolus lunatus* is best known as the causative agent of seedling blight and seed germination failure in monocotyledoncrops in such as sugarcane, rice, millet and maize *Cochliobolus lunatus* also causes leaf spot on a wide variety of angiosperm hosts, where each lesion contains a spours mass of fungi at its center. The Clk1 gene plays an important role in fungal growth during the infection process, specifically condition, which is vital to the process of foliar infection. fungicides in particular those with organo-mercurial compounds, have been associated with effective eradication of this pathogen.

**Fig 1.4 Leaf spot on maize plant**

### 1.4.4 Leaf spot disease on maizeplant:

Maize, Zea mays L. is one of the most important cereals in the world after wheat and rice with regards to cultivation area and total production. Maize is high yielding, easy to process, readily digested and cheaper than others cereals. it's also a versatile digested and cheaperthanothercereals.Itisalsoaversatilecrop,growingacrossarangeofagroecological zones. Every part of the maize plant has ecological value : the grain, leaves ,stalks ,tassel and cob can all be used to produce a large variety of food and non-foodproducts

With this important of maize, it is being plagued by an array disease which include the leaf spot of maize which is caused by *C. lunata*. This disease is a very important seed and oil borne disease prevalent in the hot ,humid maize areas. The disease produces small necrotic or chloroticspotwithalightcoloredhalo;lesionsareabout0.5cmperspotwhenfullydeveloped and this causes significant damage maize up to 60% due to great loss of photosyntheic region of thecrop

Attempts have been made to develop maize cultivars that are develop maize cultivars that are resistant to leaf spot, and many other control measures have also been used to check thisfungaldisease.Theseincludeimprovedculturalpracticesonthefarmandchemicalcontrol usingfungicidesaAndwerethenfoundtobeeffectiveagainstleafspotwhentested.But,most of these fungicides are not available to peasant farmers because most of the fungicides are expensive, require skilled labour and add to the cost of production while the yield obtained by theirusesmaynotbesufficienttojustifycostofproduction.Alsomostofthesefungicidesare toxic to humans and with the dwindling foreign exchange and prohibitive cost; most of the useful fungicides are usually out of reach of peasant farmers, The pathogens on its own, also build up resistance to the fungicides and even when resistant varieties are planted in endemic areas.

### *1.5* BIOFERTILIZER

Biofertilizers are natural fertilizes which are living microbial inoculants of bacteria, algae,fungialoneorincombinationandtheyaugmenttheavailabilityofnutrientstotheplants. Theroleofbiofertilizersinagricultureassumesspecialsignificance,particularlyinthepresent

context of increased cost of chemical fertilizer and their hazardous effects on soil health (Narendra kumaw at on Feb 15, 2018)

Sustainable agriculture development is a very important challenge that encounters the world nowadays as it requires increasing the productivity of plants with minimal disturbance of the environment. Plant growth is very susceptible to different conditions that affect its productivity and yield. These conditions could be divided into biotic (living) and abiotic (nonliving) stresses. Biotic stress includes interference from pathogenic microorganisms, insects, and higher animals, which include humans, while abiotic stress includes soil salinity, waterlogging, drought, high and low temperatures, wind, intense light, heavy metals, and inadequateorexcessivemineralnutrients.Mostoftheabioticstressfactorscouldbeattributed todifferentclimaticchangeswhichareconsideredthemajorreasonsforregressionofprincipal crop productivity. Plant species are surrounded by diverse beneficial microorganisms that dominate in their rhizosphere and have the ability to stimulate plant growth and protect them against different stress conditions. Different microbial activities have the ability to improve plant tolerance to biotic and abiotic stress conditions. The role of alleviation depends on the plant genus, the stress type, the microbial species, and the type of relationship between microorganisms and the plant. Microorganisms could enhance plant survival, growth, performance, and yield by several functions such as stimulating root growth by production of phytohormones, enabling water uptake to roots by production of polysaccharides in the root hair zone, improving plant nutrition by increasing nutrients through solubilization of phosphate,secretingsiderophoresforiron,andfixingdinitrogen,whichiseitherassociativeor nonassociative.Usingmicrobialinoculantsisconsideredanimportanttaskinthenextdecades to counter abiotic stress in different regions (Mona S. Zayed on Jun 07,2018)

### 1.5.1 Merits ofbio-fertilizers

1) A biofertilizer is a natural product carrying living microorganisms derived from the plant root or cultivated soil. As such no harmful effect on soil fertility is generallyseen.

2) Biofertilizer is required in smaller dose. A dose of 350- 500 gm. of material per hectare is often sufficient to give desirable effect. This is because each gram of carrier of biofertilizer contains at least 10 million viable cells of a specificstrain.

3) Wide variety of biofertilizers with proven utility for large number of crop species are now available in the market. Effect of biofertilizers in increasing the yield of different crops under irrigated and rainfed conditions has been proved.

4) Besides their direct effect on current crop, use of a biofertilizer also leaves considerable beneficial residual effect on sodfertility.

5)Biofertilizers may exert favourable effect on root growth and crop standby affecting general growth and development of plant. For example, *Azospirillum* and *Phosphobacterin* produce growth promotingsubstances.

6) It may hasten flowering and crop maturity to a certainextent.

7) Rhizobium culture possesses better tolerance to salt and pH under various ecological conditions, therefore, possess better adaptability to different agro- climaticsituations.

8) Use of biofertilizers is economical with a high cost: benefit ratio, withoutrisk.

9) Somebiofertilizersmayworkasbiopesticide.Forexample,*Azotobacterin*strainhasshown potential to inhibit seed borne pathogen in somecereals.

10) Biofertilizers are renewable and pollutionfree.

## *1.6 Sargassummuticum*

*Sargassum muticum*, commonly known as Japanese wireweed, is a large brown seaweedofthegenus Sargassum.Itisaninvasiveseaweedwithhighgrowthrate(upto10cm per day during spring). It has an efficient dispersion thanks to its float *Sargassum muticum* is abrownseaweed,normallybrowntoyellowishwithalengthupto10m.Itisanautotrophthat uses energy from sunlight. The photosynthesis is facilitated thanks to aerial vesicles which allows the algae to raise to thesurface.

*Sargassummuticum*iscomposedoftwodistinctparts:aperennialpart,whichcontains the holdfast and one or more short main axes; and an annual part: the secondary axes, which develop on the main axis, whose growth is unlimited and the size is variable. There are three types of ramifications: laterals with foliaceous expansions called fronds, laterals with fronds andaerocystsandlateralswithfronds,aerocystsandreproductiveorganscalledreceptacles.In winter, only the perennial part persists (5 cm). In summer, the lateral part is in maximum development of 2–3 meters to 10meters.

### 1.6.1 SEAWEED ASBIOFERTILIZER

Seaweeds are large plants growing in the sea, especially various marine algae like the rockweeds,kelps,sealettuceanddulses.Driedorfreshseaweedsandliquidextractshavebeen increasingly employed by horticulturists, gardeners, farmers, and orchadists as a fertilizer. Seaweed extracts are now commercially available as maxicrop, seasol, SM3, kelpak , and cytokine.Theeffectofseaweedextractisduetothemicroelementsandplantgrowthregulators such as cytokinin present in It(FAO, 2006). Seaweed extract is used as a foliar spray, applicationtosoilandforsoakingofseedsbeforesowing.Itenhancesthegerminationofseeds, increases uptake of plant nutrients, and gives resistance to frost and fungal diseases. Seaweed extract is effective for ripening of fruits, increasing shelf-life of the produce, improves the qualityofproduce,andservesasanexcellentsoilconditioner.Seaweedextractsarealsoknown as biological fertilizers (Zodape, 2001).

Seaweed biofertilizers contain macronutrients (Ca, Mg, P, and K), micronutrients(Fe, Cu, Zn, B, Mn, Co, and Mo), as well as various plant growth regulators (Kumar et al., 2012). Various studies have confirmed that seaweeds contain auxins or auxin-like compounds and it has been observed that *A. nodosum* contains approximately 50 mg of indole-3-acetic acid per gramofdryextract(KingmanandMoore,1982;CrouchandvanStaden,1992).Cytokinehave also been reported from seaweed extracts, containing trans-zeatin, benzyl amino purine, and topolin (Strike and van Staden, 1997; Stirk et al., 2004; Ördog et al., 2004). Extracts of *A. nodosum* also contain betaine and betaine-like compounds as well as gibberellins (Blunden et al., 1986; Craigie,2010).

### 1.6.2 Important uses of seaweeds

Increase in production of phyto - chemicals such as agar-agar, carrageen and alginate. As food for human consumption as green vegetable, salads and also in the form of jelly, jam, chocolatesandpickles.Apartfromthatseaweedsareusedasrawforcosmetics.Treatment of wastewater to reduce nitrogen- and phosphorus-containing compounds. Removal of toxic metals from industrial wastewater. Integrated aquaculture Biomass for fuel, Animal feed, Fishfeed.

# CHAPTER 2
# REVIEW OF LITERATURE

**Madhurima Dutta et al,** (**2014**) Plant materials are used throughout developed and developing countries as home remedies, over the counter drug products and raw materials for the pharmaceutical industry. Some of quality control parameters of the leaves of *Calotropis gigantea*belongingtoApocynaceaefamilywereanalyzed.*Calotropisgigantea*isanimportant Indian medicinal plant and widely used in Ayurveda for management of various diseases. Different biochemical screening has been carried out to identify the important phytoconstituents. A number of biological constituents in good yield and some have been shown to possess useful biological actions belonging mainly to phenolics, flavonoids, glycosides, alkaloids, cardiac glycosides, phytosterols. Extract of this plant possess useful antimicrobialactivities.

**Pasumarthi Brahmam et al., (2019)** The present study report the phytochemical analysis of chloroform, ethyl acetate, methanolic extracts of leaf, stem bark and root of *Acalypha indica* (L.) and *Cocculushirsutus*(L.) plants. The authentication of the plant species was done by the taxonomist. The plant part extraction was done by using soxhlet apparatus. The preliminary phytochemical screening of this extracts was conducted by following the standardmethodsforthepresenceofthealkaloids,saponins,terpenoids&steroids,flavonoids, tannins, phenolic compounds, coumarins, quinones, resins, and glycosides.Results indicated the presence of alkaloids, saponins, terpenoids & steroids, tannins, anthocyanidins, phenolic compounds, coumarins, quinones, resins and glycosides in all the plant extracts and could be used for the treatment of wounds andburns.

**B.TPawaretal.,(2011)** Extractsofthevariousplantpartslikeleaf,stem,root,fruit and seeds are found to be effective against seed-borne pathogenic fungi. The *in vitro* studies havebeenperformedbyusingcup-platemethodtoexaminetheantifungalactivityofsomeleaf extracts. Leaf extracts of 18 plants were screened against 5 seed-borne pathogenic fungi *viz. Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium moniliforme* and *Trichodermaviride*.Outof18leafextracts,9leafextractsshowedantifungalactivity.The

extractof*Azadirachtaindica*showedmaximumactivity;whileminimumactivitywasoberved with *Holoptelia integrifolia* against the fungi underinvestigation.

**Tansukh Barupal et al., (2017**) Antimicrobial activity of *Lawsonia inermis* extracts against fungus *Curvularia lunata* has been assessed. We tried six different solvents for successive extraction; the purpose was to screen out the best extract in term of its fungicidal action.Amongallsolventextracts,acetoneextractshowedgreatestpercent(70.15%)inhibition of mycelia growth of target fungi. The commonly used laboratory method, poison food techniquewasusedtoevaluateandscreenthe*invitro*antifungalactivity.Mancozebandbavist inwereusedasstandards.MinimumInhibitoryConcentration(MIC)andMinimumFungicidal Concentration (MFC) of acetone fraction of *Lawsonia inermis* were investigated against *Curvularia lunata* and phytotoxicity of best partially purified extract was observed. Ourresult shows that acetone fraction of *Lawsonia inermis* has maximum antifungal activity and can be used as a powerful fungicide against *Curvularia lunata* in treating leaf spot disease of maize plant.

**KatarzynaGodlewskaetal**.,(2016)haveinvestigatedthePlantGrowthBiostimulants Based on Different Methods of Seaweed Extraction with Water We explored two methods for obtaining aqueous extracts: boiling and soaking of Baltic seaweeds (EB and ES, resp.). Algal extractswerecharacterizedintermsofpolyphenols,micro-andmacroelements,lipidscontent, and antibacterial properties. The utilitarian properties were examined in the germination tests on Lepidium sativum for three extract dilutions (0.5, 2.5, and 10%). It was found that the extracts were similar in micro- and macro element concentrations. Water was proved to be a good solvent to extract phenolic compounds. The algal extract produced by soaking biomass did not show inhibitory effect on Escherichia coli and Staphylococcus aureus. Only the boiled extracthadaninhibitoryactivityagainstE.coli.Germinationtestsrevealedapositiveinfluence ofthebioproductsonthecultivatedplants.Inthegrouptreatedwith10%EB,plantswere13% longerthaninthecontrolgroup;thecontentofelementsB,Mo,Zn,andNainthegrouptreated with 10% ES was higher by 76%, 48%, 31%, and 59% than in the control group,respectively; the content of chlorophyll was 2.5 times higher in 0.5% ES than in the control group. Extracts showed the slight impact on the morphology ofplants.

**Tansukh Barupal et al., (2019)** have studied the antifungal activity of *Lawsonia inermis* against the fungal stain *curvularia lunata.* Effect of partially purified acetone fraction of *L. inermis* leaves on various cytomorphological parameters i.e. mycelium width, conidial

size, etc. of test fungi and fraction was subjected to confirming the presence of primary and secondarymetabolitesbyrapidqualitativephytochemicaltests,chromatographicmethodssuch as TLC, column chromatography, GC–MS, etc. which were responsible for the inhibition of growth of test pathogen conidial size of *Curvularia lunata* decreased up to 64.76% at 0.039 µg/mlconcentrationoftheextract.Mycelialwidthof*C.lunata*increasedupto55.91% at 0.312 µg/ml concentration of the extract. Carbohydrate, steroids, volatile oils, flavonoids, and tannins were found to be present in acetone extract of *L. inermis*leaf.

**Somchitetal.,(2010)**haveinvestigatedtheantimicrobialactivityofwater,ethanoland chloroform extracts of *Acalypha indica* against four bacterial and fungal strains using the disc diffusion method. The antifungal activity was more significant ($p< 0.05$) only in chloroform extract.

**Seham et al., *(*2018)** have investigated the role of marine macroalgae in plant protection.Marinemacroalgaearealsocharacterizedbyproducingalargearrayofbiologically active biocidal substances against plant-infecting pathogens. Bioactive compounds like fatty acids(inparticularpolyunsaturatedfattyacids(PUFAs),proteins(aminoacids),bioflavonoids, sulfated polysaccharides, carotenoids, polyphenols and carbohydrates are considered to have bactericidal, antiviral and fungicidal effects against some plant-infecting pathogens. These biocontrolagentsprovidemultiplebenefitsandactasusefulpointersforimprovingcultivation practices in diverse habitats. Commercial production and exploitation of specific compounds with interesting biotechnological importance from marine macroalgae includingmicrobicides, nematicides,insecticides, biofertilizers, biostimulators and soilconditioners. Marine macroalgae can be generally considered as promising multifunctional bioinoculants and ecofriendly environmental tools in recent trends of organicfarming.

**Silva et al.,** (**2019**) have studied the biofertilizer production from *Sargassummuticum*. Seaweeds produce many compounds and secondary metabolites that can be used in different fields of industry such as food, agricultural, pharmaceutical and health. Seaweed has many bioactive compounds beneficial to plant development, giving them a great potential as an agricultural fertilizer. Adding seaweeds to the soil provides organic matter, minerals, trace elements, growth plant regulator, metabolites, vitamins, and amino acids and it can work as a soil conditioner. The potential of the extracts obtained from *Ascophyllum nodosum* and from *Sargassummuticum*asanagriculturalfertilizer.Thisevaluationwascarriedoutwithriceplants (*Oryzasativa*)andlettuce(*Lactucasativa*),ingerminationbioassays,thecultureofriceand

lettuce plants in pots, and culture of lettuce plants in hydroponics. For that, seaweed liquid extractswereusedindifferentconcentrationsindifferentbioassays.Resultsshowthatextracts obtainedfromtwoseaweeds,*A.nodosum*and*S.muticum*,canbepromissoryplantbiofertilizer at a concentration of 25% and had a positive effect on seed germination, plant development, andproduction.

**Mohamed et al., (2019)** have investigated the Red sea stretches along Marsa Alam of Egypt is a habitation of diverse groups of marine macro-algal species recorded along Marsa Hemerashore.Ecologicalstudies(meteorologicaldata,water,soil,aqueousextractanalysisof *Sargassum muticum* (Yendo) Fensholt and associated species) reported that, slightly alkaline; low turbidity, moderate temperature and available nutrient content of saline water produced massive growth of ***S. muticum*** during September (2018). Ecological and Physico-chemical properties of collected water samples showed variations of different parameters of sea water like temperature, salinity, pH, and high dissolved minerals. Heavy metals accumulation inside theinvestigatedseaweeds***S.****muticum*waswithinthecorrespondingrange.Theaqueousextract of ***S. muticum*** contained high amounts of Na, Ca, K and Fe, moderate amounts of Zn, and low in Cu, Cd, Ni and Mn. Lead Pb recorded 0.291 ppm, Ag, Co and Ga were absent in aqueous extract. The associated species to the brown algae ***S. muticum*** are belonging to 6 families and 7 species as follows: *Sargassaceae, Fucaceae, Phaeophyceae, Rhodomelaceae,Caulerpaceae* and *Hydrocharitaceae*. Results showed that ***S. muticum*** enriched in essential amino acids; micro and macro elements, carbohydrates, protein, lipids, and agar. These algae may be used as biofertilizers.

**Pawarnt et al**., **(2018)** have investigated the antifungal activity of *Calotropisgigantea* leaf extract, It was found to be effective against seed-borne pathogenic fungi. The *in-vitro* studieshavebeenperformedbyusingcup-platemethodtoexaminetheantifungalactivityof *C. gigantea* leaf extract. It was screened against 5 seed-borne pathogenic fungi *viz. Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium moniliforme* and *Trichoderma viride.* Out of them, antifungal activity of *C. gigantea* leaf extract against *C. lunata* was found maximum(Meanactivityzone-19.33mm)followedby*A.alternata*(Meanactivityzone-17.67mm);whileminimumactivitywasobservedagainst*A.niger*(Meanactivityzone-14.67 mm). *C. gigantea* leaf extract can possibly be exploited in the management of seed-borneppathogenic fungi to prevent biodeterioration of seeds in an eco-friendly way.

# Chapter 3
# Materials and Methods

## 3.1 COLLECTION AND PROCESSING OF PLANTMATERIALS:

The Leaves of *Acalypha indica*(kuppaimeni), *Lawsonia inermis*(maruthani) and *Calotropis gigantea*(erukan) were collected from Putlur village, Thiruvallur taluk. The collected leaf of all the three plants were washed thoroughly with distilled water and the plant material were shade dried for two weeks. The Shade dried leaves of all the three plants was grounded using mortar and pestle to powder. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

## 3.2 COLLECTION OFORGANISM:

FungalstrainCurvularialunatawascollectedfromBiozoneResearchTechnologiesPvt.Ltd., Zameen Pallavaram.

**Fig 3.1*: Curvularia lunata***

## 3.3 STERILIZATION:

Media and glassware were sterilized in autoclave at 15psi pressure at for 121°-C 0min

## 3.4 PREPARATION OFINNOCULUM:

10ml of potato dextrose agar prepared, to that fungus *Curvularia lunata* was inoculated and incubated for 4 days at 30°-C in incubator.

**Fig 3.2: Innoculum of *Curvularia lunata***

## 3.5 SEQUENTIALEXTRACTION:

Different solvents are choose with different polarity to get respective phytochemicals present in the plant species (leaves of *Acalypha indica, Lawsonia inermis* and *Calotropis*).

### 3.5.1 EQUIPMENTS:

Weighing balance, laminar air flow chamber, incubator, autoclave, freezer, Soxhlet apparatus.

### 3.5.2 REQUIREMENTS:

All the requirements are maintained in aseptic condition, Petroleum ether, Chloroform, Methanol, Conical flask, Soxhlet apparatus.

### 3.5.3 SOXHLETEXTRACTION:

Soxhlet extractor is a kind of laboratory equipment used for solid-liquid extraction. Normally a solid material containing some of the desired compound was placed inside a thimble made from thick filter paper, which was loaded into the main chamber of the soxhlet extractor. The soxhlet extractor was placed onto the flask containing the extraction solvent, The soxhlet was then equipped with a condenser.

The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and the floodintothechamberhousingthethimbleofthesolid.Thecondenserensuresthatanysolvent vapours cools and drips back down into the chamber housing the powderedsample.

The chamber containing the sample slowly fills with the warm solvents. Some of the desired compoundwillthendissolveinthewarmsolvent.Whenthesoxhletchamberisalmostfull,the

chamber is automatically emptied by a siphon side arm, with the solvents back down to the distillation flask. This cycle may be allowed to repeat many times over 5 hours.

During each cycle, a portion of the non-volatile compound dissolves in the solvents. After many cycles the desired compound was concentrated in the distillation flask.

After extraction the solvent was removed, typically by means of a rotary evaporator, yielding theextractedcompound.Thenon-solubleportionoftheextractedsolidremainsinthethimble, and was usuallydiscarded.

## 3.5.4 PETROLEUM ETHER, CHLOROFORM AND METHANOL EXTRACT OF *Acalypha indica* LEAF: PREPARED USING SOXHLET EXTRACTOR

### *3.5.4.1* PETROLEUM ETHER EXTRACTION OF *Acalypha indicaLEAF:*

Thefinelygroundpowder(80gm)wasplacedinaporousbagorthimblemadeoutofstrong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatus isplacedontoaroundbottomflaskcontainingtheextractingsolventpetroleumether(300ml). Thesolventsareusedintheincreasingorderofpolarity.Theextractingsolventpetroleumether (300ml) in the flask was heated at its boiling point, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the plant powder and extract it by contact. When the level of the liquid in the chamber have reached the top of siphon tube, the liquidcontentofthechambersip-honintotheflask.Afterextractionthesolventwasremoved, typically by evaporation, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and was usually discarded. The obtained extract was condensed for furtheruse.

### *3.5.4.2* CHLOROFORM EXTRACTION OF *Acalypha indica*LEAF:

The finely ground powder (80gm) after the treatment with the petroleum ether (low polar solvent) the powder was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatus is placed on to a round bottom flask containing the extracting solvent chloroform (300 ml). The solvents are used in the increasing order of polarity. The extracting solvent chloroform (300ml) in the flask was heated at its boiling point, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the plant powder and extract it by contact. When

thelevelofftheliquidinthechamberhavereachedthetopofsiphontube,theliquidcontentof the chamber sip-hon into the flask. After 72 hours the dried compound is concentrated in the flask. After extraction the solvent was removed, typically by evaporation, yielding the extractedcompound.Thenon-solubleportionoftheextractedsolidremainsinthethimble,and was usually discarded. The obtained extract was condensed for furtheruse.

### *3.5.4.3* METHANOL EXTRACTION OF *Acalypha indica*LEAF:

The finely ground powder (80gm) after the treatment with chloroform, the powder was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatus is placed on to a round bottom flask containing the extracting solvent methanol (300 ml). The solvents are used in the increasing orderofpolarity.Theextractingsolventmethanol(300ml)intheflaskwasheatedatitsboiling point,anditsvaporscondenseinthecondenser.Thecondensedextractantdripsintothethimble containing the plant powder and extract it by contact. When the level of the liquid in the chamber have reached the top of siphon tube, the liquid content of the chamber sip-hon into the flask. After 72 hours the dried compound is concentrated in the flask. After extraction the solvent was removed, typically by evaporation, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and was usually discarded. The obtained extract was condensed for furtheruse.

## 3.5.5 PETROLEUM ETHER, CHLOROFORM AND METHANOL EXTRACT OF *Calotropis gigantea* LEAF – PREPARED USING SOXHLETEXTRACTOR

### *3.5.5.1* PETROLEUM EXTRACTION OF *Calotropis gigantea*LEAF:

The finely ground powder (80gm) was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatusisplacedontoaroundbottomflaskcontainingtheextractingsolventpetroleumether (300 ml). The solvents are used in the increasing order of polarity. The extracting solvent petroleumether(300ml) intheflaskwasheatedatitsboilingpoint,anditsvaporscondensein the condenser. The condensed extractant drips into the thimble containing the plant powder and extract it by contact. When the level of the liquid in the chamber have reached the top of siphon tube, the liquid content of the chamber sip-hon into the flask. After extraction the solventwasremoved,typicallybyevaporation,yieldingtheextractedcompound.Thenon-

soluble portion of the extracted solid remains in the thimble, and was usually discarded. The obtained extract was condensed for further use.

### *3.5.5.2* CHLOROFORM EXTRACTION OF *Calotropis gigantea*LEAF:

The finely ground powder (80gm) after the treatment with the petroleum ether (low polar solvent) the powder was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatus is placed on to a round bottom flask containing the extracting solvent chloroform (300 ml). The solvents are used in the increasing order of polarity. The extracting solvent chloroform (300ml) in the flask was heated at its boiling point, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the plant powder and extract it by contact. When thelevelofftheliquidinthechamberhavereachedthetopofsiphontube,theliquidcontentof the chamber sip-hon into the flask. After 72 hours the dried compound is concentrated in the flask. After extraction the solvent was removed, typically by evaporation, yielding the extractedcompound.Thenon-solubleportionoftheextractedsolidremainsinthethimble,and was usually discarded. The obtained extract was condensed for furtheruse.

### *3.5.5.3* METHANOL EXTRACTION OF *Calotropis gigantea*LEAF:

The finely ground powder (80gm) after the treatment with chloroform, the powder was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatus is placed on to a round bottom flask containing the extracting solvent methanol (300 ml). The solvents are used in the increasing orderofpolarity.Theextractingsolventmethanol(300ml)intheflaskwasheatedatitsboiling point,anditsvaporscondenseinthecondenser.Thecondensedextractantdripsintothethimble containing the plant powder and extract it by contact. When the level of the liquid in the chamber have reached the top of siphon tube, the liquid content of the chamber sip-hon into the flask. After 72 hours the dried compound is concentrated in the flask. After extraction the solvent was removed, typically by evaporation, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and was usually discarded. The obtained extract was condensed for furtheruse.

## 3.5.6 PETROLEUM ETHER, CHLOROFORM AND METHANOL EXTRACT OF *Lawsonia inermis* LEAF – PREPARED USING SOXHLET EXTRACTOR.

### *3.5.6.1* PETROLEUM ETHER EXTRACTION OF *Lawsoniainermis* LEAF:

Thefinelygroundpowder(80gm)wasplacedinaporousbagorthimblemadeoutofstrong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatus isplacedontoaroundbottomflaskcontainingtheextractingsolventpetroleumether(300ml). Thesolventsareusedintheincreasingorderofpolarity.Theextractingsolventpetroleumether (300ml) in the flask was heated at its boiling point, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the plant powder and extract it by contact. When the level of the liquid in the chamber have reached the top of siphon tube, the liquidcontentofthechambersip-honintotheflask.Afterextractionthesolventwasremoved, typically by evaporation, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and was usually discarded. The obtained extract was condensed for furtheruse.

### *3.5.6.2* CHLOROFORM EXTRACTION OF *Lawsonia inermis*LEAF:

The finely ground powder (80gm) after the treatment with the petroleum ether (low polar solvent) the powder was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatus is placed on to a round bottom flask containing the extracting solvent chloroform (300 ml). The solvents are used in the increasing order of polarity. The extracting solvent chloroform (300ml) in the flask was heated at its boiling point, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the plant powder and extract it by contact. When thelevoftheliquidinthechamberhavereachedthetopofsiphontube,theliquidcontentof the chamber sip-hon into the flask. After 72 hours the dried compound is concentrated in the flask. After extraction the solvent was removed, typically by evaporation, yielding the extractedcompound.Thenon-solubleportionoftheextractedsolidremainsinthethimble,and was usually discarded. The obtained extract was condensed for furtheruse.

### *3.5.6.3* METHANOL EXTRACTION OF *Lawsonia inermis*LEAF:

The finely ground powder (80gm) after the treatment with chloroform, the powder was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The soxhlet apparatus is placed on to a round bottom flask containing the extracting solvent methanol (300 ml). The solvents are used in the increasing

orderofpolarity.Theextractingsolventmethanol(300ml)intheflaskwasheatedatitsboiling point,anditsvaporscondenseinthecondenser.Thecondensedextractantdripsintothethimble containing the plant powder and extract it by contact. When the level of the liquid in the chamber have reached the top of siphon tube, the liquid content of the chamber sip-hon into the flask. After 72 hours the dried compound is concentrated in the flask. After extraction the solvent was removed, typically by evaporation, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and was usually discarded. The obtained extract was condensed for furtheruse.

**Fig:3.3 Extraction of *Acalypha indica*, *Lawsonia inermis* and *Calotropis gigantea* leaves using soxhlet extractor**

## *3.6* QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *Acalypha indica*, *Calotropis* and *Lawsoniainermis*

### 3.6.1 TEST FORALKALOIDS:

**MAYER'S TEST**: 2ml of plant extract was dissolved with 2ml of concentrated hydrochloric acid and then few drops of mayer's reagent was added. Presence of green colour or white precipitate indicates the presences ofalkaloid.

### 3.6.2 TEST FORGLYCOSIDES:

To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates the presence of glycosides.

### 3.6.3 TEST FORFLAVANOIDS:

**ALKALINEREAGENTTEST**:2mlofplantextractandwasdissolvedin1mlof2Nsodium hydroxide. Presences of yellow colour indicates the presence offlavonoids.

### 3.6.4 PHENOLIC TEST:

1mlofplantextractandfewdropsofphenol-densreagentwereaddedfollowedby2mlof15% sodiumcarbonatesolution.Formationofblueorgreencolourindicatesthepresenceofphenols.

### 3.6.5 TEST FORTERPENOID:

**SALKOWSKI TEST**: 0.5 ml of plant extract and 2ml of chloroform and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids.

### 3.6.6 TEST FORTANNIN:

**FOLIN-DENISREAGENTTEST**:1mlofplantextractwasdissolvedwith2mlof7MNaOH andthenfewdropsoffolin-denisreagentwereadded.Formationofdarkblueorgreenishblack indicates the presences oftannins

### 3.6.7 TEST FORQUINONES:

**CONCENTRATED SULPHURIC ACID TEST**: 1ml of plant extract was dissolved with 1ml of concentrated sulphuric acid. Formation of red colour indicates presence of quinines.

### 3.6.8 TEST FORSAPONINS:

**FROTHTEST**:2mlofplantextractswasdissolvedwith2mlofdistilledwaterandthenshaken in a graduated cylinder for 15 minutes length wise. Formation of 1cm layer of foam indicates the presences ofsaponins.

### 3.6.9 TEST FORCARBOHYDRATES:

**MOLISCH'S TEST**: 2 ml of plant extract was dissolved with 1ml of Molisch's reagent and few drops of concentrated sulphuric acid was added to the extract which produced a reddish colour or purple colour indicates the presence of carbohydrates.

### 3.6.10 TEST FOR CARDIACGLYCOSIDES:

0.5 ml of plant extract was dissolved with 2ml of glacial acetic acid and added few drops of 5%ferricchloride.Thiswasunderlayeredwith1mlofconcentratedsulphuricacid.Formation of brown ring at the interface indicated the presence of cardiacglycosides

### 3.6.11 TEST FORCOUMARINS:

To1mlofextract,1mlof10%NAOHwasadded.formationofyellowcolorindicatespresence ofcoumarins.

### 3.6.12 TEST FORSTEROIDS:

1ml of plant extract and equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicated the presence of steroids and appearances of bluish brown ring indicated the presence of phyto steroids.

### 3.6.13 TEST FORPHLOBATANNIS:

1mlofplantextractandfewdropsof2%HCLwasaddedappearanceofredcolourprecipitate indicates the presence of phlobatannins.

### 3.6.14 TEST FORANTHRAQUINONES:

1ml of plant extract and few drops of 10% ammonia solution were added, appearance of pink colour precipitate indicated the presence of anthraquinones.

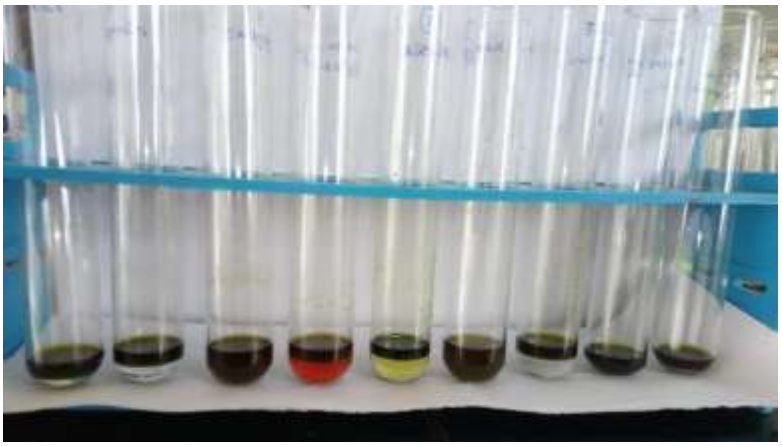


**Fig:3.4 Screening of phytochemicals of *Acalypha indica* leaves extract**

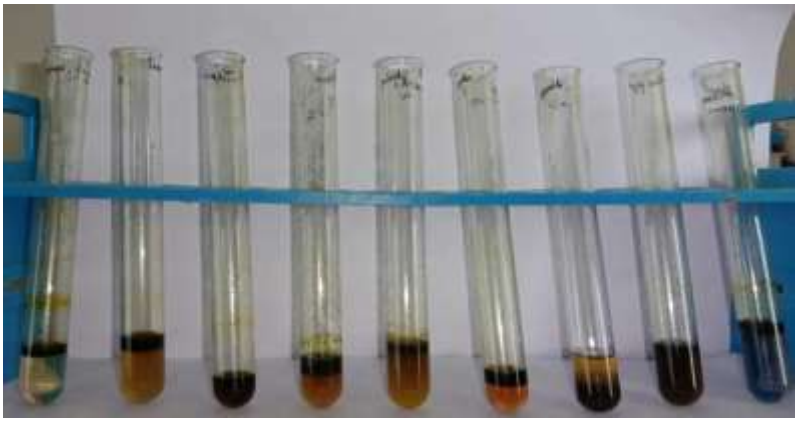


**Fig:3.5 Screening of phytochemicals of *Lawsonia inermis* leaves extract**

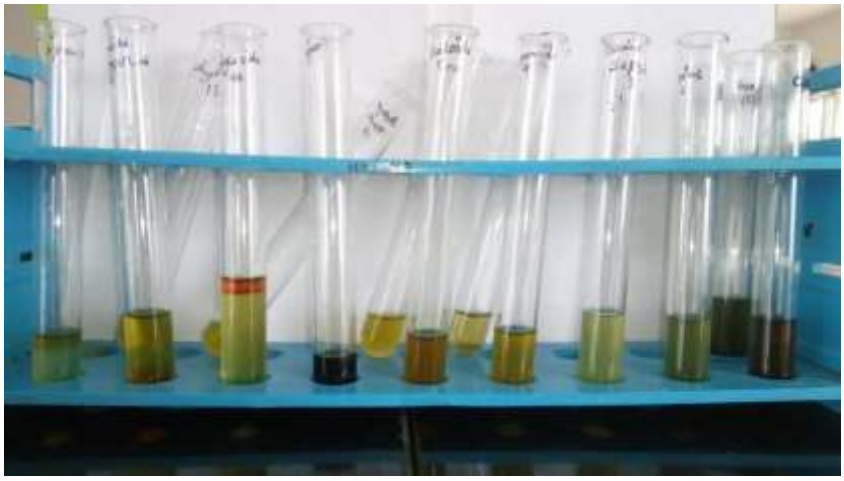


**Fig:3.6 Screening of phytochemicals of *Calotropis gigantea* leaves extract**

## 3.7 QUANTITATIVE PHYTOCHEMICALANALYSIS:

### 3.7.1 DETERMINATION OF FLAVONOIDS CONTENT:

Total Flavonoids content in the extracts was determined using the method described (Sankanaka, et a*l*, 2005).The flavonoids content was determined by aluminium chloride method using Quercetin as standard. Extract and quercetin were prepared in Ethyl acetate (10mg\ml). 0.1ml of extract was mixed with 0.9ml of distilled water in test tube, followed by

additionof75µlof5%sodiumnitritesolution.150µlof10%aluminiumchloridesolutionwas added after 6 minutes and the mixture was allowed to stand for further 5 minutes after which 0.5ml of 1M sodium hydroxide was added to the reaction mixture .The reaction mixture was brought to 2.5 ml distilled water and mixed well. The absorbances was measured immediately at 510 nm using a spectrophotometer. A calibration curve was generated using various concentration of Quercetin (20-120g). Blank consists of all the reagents, except for the extract or Quercetin is substituted with 0.1 ml of ethanol. The result was expressed as the Quercetin
equivalences(QE)ofthesampleinmg\mloftheextract.Totalcontentofflavonoidscompound was calculatedas.

**Total flavonoids content=QE*V/M**

Where QE, is the Quercetin equivalences (mg\ml) or concentrated solution of quercetin established from calibration curve, V is the volume of extract (ml) and m is the weight of the pure plant extract (g).

## 3.7.2 DETERMINATION OF TOTAL PHENOLICCONTENT:

The amount of phenolic compounds in the extracts was determined by the Folin Ciocalteu colorimetric method and calculated from a calibration curve obtained with Gallic acid as standard(1mg/1ml).Fromthestandardsolution20to100mlwasaddedtodifferenttesttubes. Extractwasaddedinaseparatetesttubeataconcentrationof1mg/ml.To0.1mlofeachextract, 5ml of follin-ciocalteu (1:10 dilution) was added and the content was mixed thoroughly and incubated in dark for 3minutes. 5ml of sodium carbonate (75g/l) was added and the mixture was incubated in dark for 60 min. The absorbance was measured at 765nm in a UV –visible spectrophotometer. They were expressed in gallic acid equivalence of the samples (GE) g/mg of the extract .Total contents of phenolic compound is calculatedas

**Total phenolic content =GAE * V/m,**

Where GAE, is the gallic acid equivalence (mg/ml) or concentration of gallic acid solution established from the calibration curve V, is the volume of extract (ml) and m, is the weight of the pure plant extract (g).

## 3.8 INVITRO ANALYSIS OF ANTIFUNGAL ACTIVITY OF *Acalypha indica, Calotropis gigantea* and *Lawsonia inermis* LEAF EXTRACT BY AGAR WELL DIFFUSIONMETHOD:

Well diffusion method was adopted for evaluation of antifungal activity of three different medicinal leaves. Agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled at 45°C. the cooled media was poured on to the sterile petriplates and allowed for solidification. The plates with media were seeded with the respective fungus suspension using sterileswab.TheWellimpregnatedwithrespectiveleafextractatdifferentconcentration(100-300mg/ml)individuallywereplacedonthefourcornersofeachpetridishes,fluconazolewere placed in the control well. The petridishes were then incubated at 37° C for 24 hours. After incubation period, the diameter of the zone formed around the well weremeasured.

## 3.9 BIOFERTILIZER PREPARATION:

### 3.9.1 Collection ofsample:

The Seaweed *Sargassum muticum* were collected from Rameswaram, Tamil Nadu.Seaweed samples were handpicked and immediately washed with seawater to remove foreign particles, sand particles and epiphytes. The samples were kept in polythene bags with seawater and immediately transported to Biozone research laboratory for the identification of the species. Then washed thoroughly using tap water to remove the salt on the surface of the sample and finally with distilled water. Then seaweeds were spread on blotting papers to remove excess water and it is placed in spread in white cotton cloth for the process of shade drying.

**Fig 3.7 *Sargassum muticum***

### 3.9.2 Extraction of seaweed liquidfertilizer:

The100gmofchopped*Sargassummuticum*wastakenanditwasblendedfor20minutesusing motor and pestle for a clear grinded extraction. Then the powdered mixture was taken in a 1000mlconicalflaskand500mlofwaterisaddedintheconicalflaskandtheflaskisboiledat 100°C for 30 minutes. This process yielded 500ml of concentrated extract of *Seaweed.* ConcentratedextractwasfilteredwithWhatmanfilterpapertoremovedebris.Thenthefiltered extract is taken and centrifuged and supernatant is taken as the concentrated liquid fertilizer. For the concentrated extract, 0.1% of formaldehyde solution (5ml) was added to preserve the extract. The Seaweed liquid fertilizer (SLF) were prepared by adding distilled water. Finally the concentrated seaweed extract was taken in falcon tube and stored for future use at 4. **(Katarzyna Godlewska et al.,(2016)**

### 3.9.3 Selection of crop plant:

The crop plant, selected for the present study was maize plant. The seeds were collected from provisional store located in Maraimalainagar. The selected seeds were stored in a plastic container until use in the project.

### 3.9.4 Preparation of crude extract

For the polyherbal formulation, 300 gm of *Lawsonia inermis* leaf was taken in the soxhlet extractor under chloroform as a solvent and the soxhlet was performed for several times until the colour change.

### 3.9.4 Culture ofseed:

Totally3Plastictrayswereusedforraisingthecrops.Thetrayswerefilledwithequalamount ofsoilfilledinthetrayof3kg.Then60maizeseedsaretakenand20maizeseedsweresoaked in 1% of seaweed extract and 10% crude extract of *Lawsoina inermis* chloroform extract.And another 20 maize seeds were soaked in 5% of seaweed extract and 10% of crude extract of *Lawsonia inermis* chloroform extract. And last 20 maize seeds were soaked in 10% seaweed extract and 10%crude extract of *Lawsoina inermis* chloroform extract for 24 hours .they were kept in a net house to prevent damage until germination**. (Jayasinghe PS, Pahalawattaarachchi(2016).**Thetrayswererearrangedatregularintervalstoensureuniform environment impact on the plant growth .The fertilizer sprays and watering were done at two daysintervals.

# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 QUALITATIVE ANALYSIS OF LEAFEXTRACTS

The phytochemical analysis confirms the presence of compounds like carbohydrate, saponins, tannins, flavonoids, quinones, steroids, alkaloids, glycosides. The presence or absence of different compounds is identified by characteristics changes when the leaf extracts treated with specific chemical reagents. The results are given in the tables.

## Table 4.1.1: PHYTOCHEMICAL ANALYSIS OF *Acalypha indica* LEAF EXTRACTS

| PHYTOCHEMICAL TEST | Petroleum ether | Chloroform | Methanol |
|---|---|---|---|
| Carbohydrates | + | + | + |
| Tannins | - | + | - |
| Saponins | - | + | - |
| Flavonoids | + | + | - |
| Alkaloids | + | + | + |
| Quinones | - | + | - |
| Glycosides | - | - | - |
| Cardiac glycosides | + | + | + |
| Terpenoids | - | + | - |
| Phenols | + | + | + |
| Coumarins | - | - | + |
| Steroids | - | + | - |
| Phlobatannins | - | - | - |
| Anthraquinones | - | - | - |

**(+ indicates presence, - indicates absence)**

**Table4.1.2:PHYTOCHEMICALANALYSISOF*Calotropisgigantea*LEAF EXTRACTS**

| PHYTOCHEMICAL TEST | Petroleum ether | Chlorofom | Methanol |
|---|---|---|---|
| Carbohydrates | + | + | + |
| Tannins | - | - | - |
| Saponins | - | - | - |
| Flavonoids | + | - | + |
| Alkaloids | + | + | + |
| Quinones | - | + | - |
| Glycosides | - | - | - |
| Cardiac glycosides | + | + | + |
| Terpenoids | - | - | - |
| Phenols | - | + | + |
| Coumarins | - | - | + |
| Steroids and Phyto steroids | - | - | - |
| Phlobatannins | - | + | - |
| Anthraquinones | - | + | - |

**Table 4.1.3: PHYTOCHEMICAL ANALYSIS OF *Lawsonia inermis* LEAF EXTRACTS**

| PHYTOCHEMICAL TEST | Petroleum ether | Chloroform | Methanol |
|---|---|---|---|
| Carbohydrates | + | + | + |
| Tannins | + | + | - |
| Saponins | - | - | - |
| Flavonoids | - | + | - |
| Alkaloids | + | + | + |
| Quinones | - | - | - |
| Glycosides | - | - | + |
| Cardiac glycosides | + | + | - |
| Terpenoids | + | + | + |
| Phenols | + | + | + |
| Coumarins | + | + | - |
| Steroids | - | + | - |
| Phlobatannins | - | - | - |
| Anthraquinones | - | + | - |

## 4.1.4 Phytochemicalanalysis

The *Acalypha indica*, *Lawsonia inermis* and *Calotropis gigantea* extract have the presenceof carbohydrates, alkaloids, flavonoids, phenols, cardiac glycosides, quinones, tannins, phytotannins,anthraquinones.

## QUANTITATIVE TEST:

### 4.1.5 DETERMINATION OF TOTAL FLAVONOIDSCONTENT

Flavonoids are the classes of polyphenolic compounds which have antioxidant properties. Flavonoids have been reported to exert multiple biological compounds which include antimicrobial, cytotoxicity study etc. The flavonoids content in petroleum ether of *Acalypha indica* leaf extract was found to be 6.86g and in the chloroform extract was foundto be 8.61g. The flavonoids content was not found in the methanol extract. The chloroform extract has the high content of flavonoids. And the flavonoids content in petroleum ether of *Calotropis gigantean* extract was found to be 6.86g and in the methanol extract was found to be23.5g.Theflavonoidscontentwasnotfoundinthechloroformextract.Themethanolextract has the high content of flavonoids. And the flavonoid content in chloroform of *Lawsonia inermis* leaf extract was found to be35.01g.

## FLAVONOID ESTIMATION:

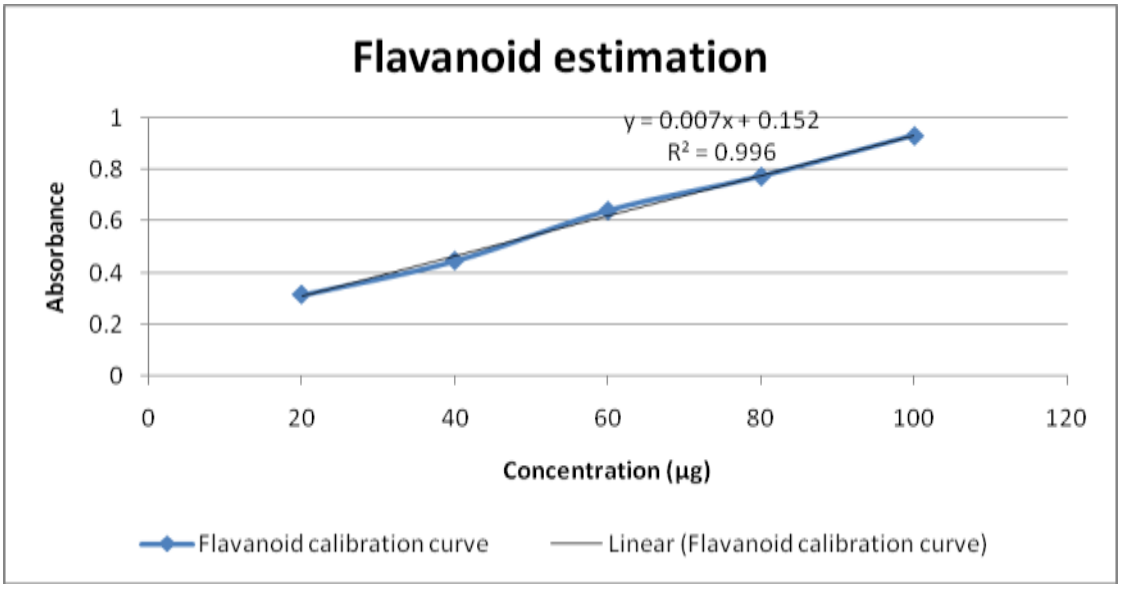



**Fig4.1: Graph shows the standard calibration curve of Quercetin**

### 4.1.6 DETERMINATION OF TOTAL PHENOLICCONTENT

Phenolic compounds are the secondary metabolitesthat are being extensively studied in plant parts of medicinal value, fruits and vegetables. The phenolic content in petroleum ether of *Acalypha indica* leaf extract was found to be 15.53g, Chloroform extract was found to be 21.03gandthemethanolicextractwasfoundtobe11.01g.ThephenoliccontentinChloroform of*Calotropisgigantea*leafextractwasfoundtobe5.256gandmethanolextractwas foundto be22.04g.Thephenoliccontentinpetroleumetherof*Lawsoniainermis*leafextractwasfound to be 112.30g, Chloroform extract was found to be 143.07g and the methanolic extract was found to be10.74g.

## PHENOL ESTIMATION:

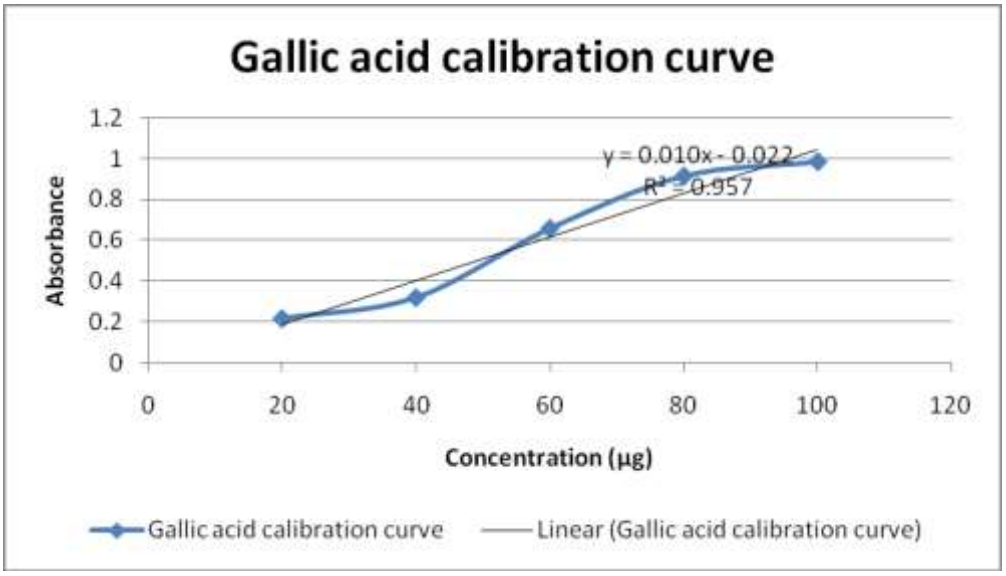



**Fig4.2: Graph shows the standard calibration curve of Gallic acid**

## 4.2 *INVITRO* ANALYSIS OF ANTIFUNGALACTIVITY

Petroleum ether, of Chloroform extract of *Acalyphaindica* exhibited maximum zone of inhibition [fig] of 12mm and 20mm against *Curvularia lunata* concentration of 80 µg /ml.

**Table4.2.1: Petroleum ether, chloroform and methanol extract of *Acalypha indica* Against *Curvularia lunata***

| EXTRACT | ZONE OF INHIBITION(mm) | | | | ANTIBIOTIC FLUCONAZOLE (1mg/ml) |
|---|---|---|---|---|---|
| | CONCENTRATION(µg/ml) | | | | |
| | 80 | 60 | 40 | 20 | |
| PETROLEUM ETHER | 12 | 9 | 6 | - | 19 |
| CHLOROFORM | 20 | 9 | 10 | 9 | 25 |
| METHANOL | - | - | - | - | 15 |

Petroleum ether, Chloroform of *Acalypha indica* exhibited maximum zone of inhibition [fig 4.3] and [fig 4.4] 12mm, 20mm against *Curvularia lunatia* at concentration of 80 µg/ml.

**Fig4.3:petroleumether**

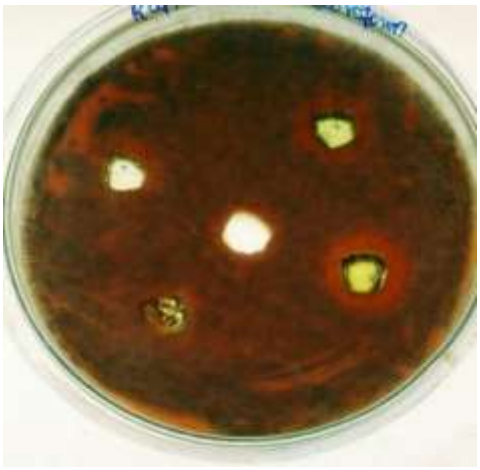


**Fig4.4:Chloroform**

**Table4.2.2: Petroleum ether, chloroform and methanol extract of *Calotropis gigantea* Against *Curvularia lunata***

| **EXTRACT** | **ZONE OF INHIBITION(mm)** | | | | **ANTIBIOTIC** FLUCONAZOLE **(1mg/ml)** |
|---|---|---|---|---|---|
| | **CONCENTRATION(µg/ml)** | | | | |
| | 80 | 60 | 40 | 20 | |

| | | | | | |
|---|---|---|---|---|---|
| **PETROLEUM ETHER** | - | - | - | - | 19 |
| **CHLOROFORM** | - | - | - | - | 15 |
| **METHANOL** | 20 | 18 | 17 | 17 | 25 |

Methanol extract of *Calotropis gigantean* exhibited maximum zone of inhibition (fig)4.5 of 20mm against *Curvularia lunata at* concentration of 80 µg /ml.

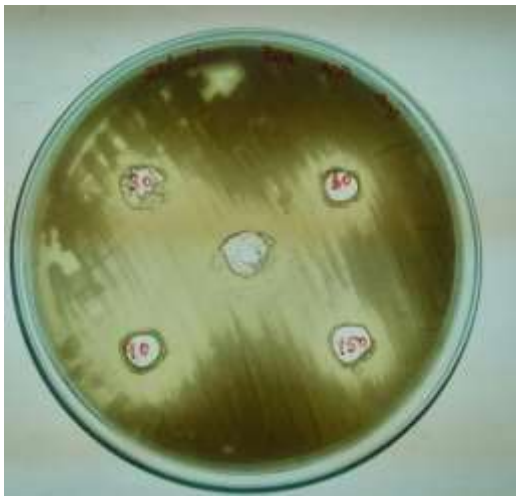
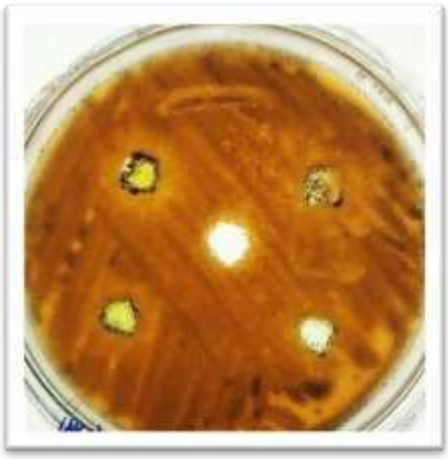


**Fig 4.5: Chloroform**

**Table4.2.3: Petroleum ether, chloroform and methanol extract of *Lawsonia inermis* Against *Curvularia lunata***

| **EXTRACT** | **ZONE OF INHIBITION(mm)** | | | | **ANTIBIOTIC** FLUCONAZOLE **(1mg/ml)** |
|---|---|---|---|---|---|
| | **CONCENTRATION(µg/ml)** | | | | |
| | 80 | 60 | 40 | 20 | |
| **PETROLEUM ETHER** | 14 | 11 | 10 | 7 | 25 |
| **CHLOROFORM** | 24 | 20 | 11 | 5 | 18 |
| **METHANOL** | 15 | 11 | 10 | - | 33 |

Petroleum ether, chloroform and Methonal extract of *Lawsoina inermis* exhibited maximum zone of inhibition of 14mm,24mm,15mm against *Curvularia lunata* at concentration of 80 µg /ml .



**4.6:petroleumether**

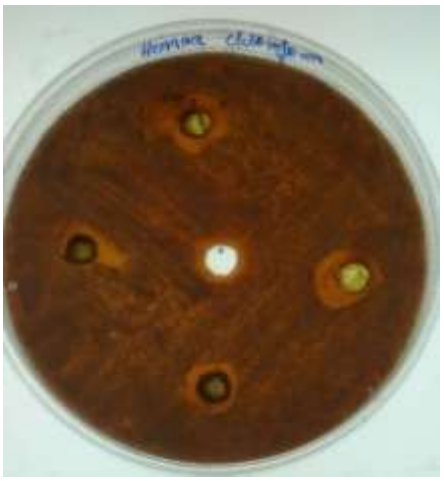


**Fig4.7:chloroform**

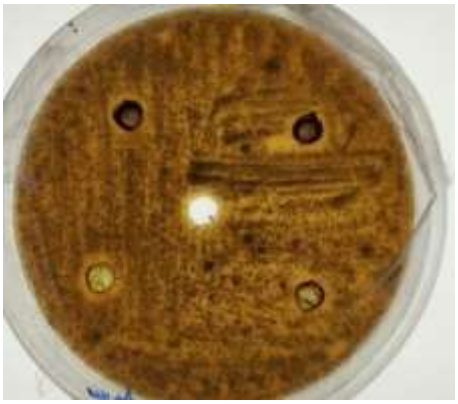


**Fig4.8: Methanol**

## 4.3 Formulation of polyherbal enriched seaweed biofertilizer:

From the antifugal activity of all the three plant leaves *Acalypha indicia, Calotropis gigantea* and *Lawsoniainermis*,Chloroform extractof*Lawsoniainermis*shows75%inhibitionofplant fungal pathogen *Curvularia lunat*a. The chloroform extract of *Lawsonia inermis* was mixed with the seaweed biofertilizer at 10%concentration.

## 4.4 : Plant growthparameters:

Totally3Plastictrayswereusedforraisingthecrops.Thetrayswerefilledwithequalamount of soil filled in the tray of 3kg. Then 60 maize seeds are taken, In treatment 1, 20 maize seeds were soaked in 1% of seaweed extract and 10% chloroform extract of *Lawsoina inermis* leaf. Andintreatment2,20maizeseedsweresoakedin5%ofseaweedextractand10%chloroform extract of *Lawsonia inermis* leaf. And last in treatment 3, 20 maize seeds were soaked in 10% seaweed extract and 10%chloroform extract of *Lawsoina inermis* leaf for 24 hours. After 24 hours the pre-treatment seeds was germinated, Plants taken from the trays were uprooted carefully after 15th day and was separated by hand picking and the following growth parameters were measured. During 15days the plant was continuously sprayed with biofertilizer.Theplantsfromeachtreatmentofdifferentconcentrationweretakenfordifferent analyses.

### 4.4.1 : Root and Shoot length of the plant of treatment1

**Extract 1- 1%concentration of seaweedextract and 10% chloroform extract of *Lawsonia inermis* leaf**

**Treatment 1**

| S.No | Root length (cm) | Shoot length (cm) |
|---|---|---|
| 1 | 31 | 16 |
| 2 | 30.9 | 15.9 |
| 3 | 29.7 | 14.5 |
| 4 | 30.8 | 15.8 |
| 5 | 29 | 14.8 |
| 6 | 29.1 | 15 |
| 7 | 28.9 | 14.9 |
| 8 | 29.2 | 15.8 |
| 9 | 28.5 | 14.8 |
| 10 | 29.1 | 14.1 |

The maximum root length of the plant grown in treatment 1 (i.e) seed soaked + fertilizer sprayed at 1% concentration of seaweed extract and 10% chloroform extract of *Lawsonia inermis* leaf is **31cm** and maximum shoot length is found to be **16cm**.

Fig 4.9 : Representative image of the uprooted plant from the treatment 1 for which the root and shoot length was measured.

## 4.4.2 :Root and Shoot length of the plant of treatment2:

**Extract2- 5%concentrationofseaweedextractand10%chloroformextractof *Lawsoina inermis* leaf**

**Treatment 2:**

| S.No | Root length (cm) | Shoot length (cm) |
|---|---|---|
| 1 | 33.1 | 18.2 |
| 2 | 32.9 | 18.1 |
| 3 | 33.1 | 18 |
| 4 | 30.6 | 17.5 |
| 5 | 30.3 | 17.9 |
| 6 | 30.1 | 16.9 |
| 7 | 29.9 | 13.1 |
| 8 | 29 | 16.8 |
| 9 | 29.7 | 15.4 |
| 10 | 28.7 | 15.5 |

The maximum root length of the plant grown in treatment 2(i.e) seed soaked + fertilizer sprayed at 5% concentration of seaweed extract and 10% chloroform extract of *Lawsonia inermis* leaf is **33.1cm** and maximum shoot length is found to be **18.2cm.**

Fig4.10: Representative image of the uprooted plant from the treatment 2 for which the root and shoot length was measured.

### 4.4.3 : Root and Shoot length of the plant of treatment3:

**Extract 3- 10% concentration of seaweed extract and 10% chloroform extract of *Lawsoina inermis* leaf**

**Treatment 3**

| S.No | Root length (cm) | Shoot length (cm) |
|---|---|---|
| 1 | 36.5 | 20.2 |
| 2 | 36.1 | 20.1 |
| 3 | 35.9 | 18 |
| 4 | 35.6 | 18.5 |
| 5 | 35.3 | 18.9 |
| 6 | 34.1 | 17.9 |
| 7 | 30.9 | 13.1 |
| 8 | 31 | 16.8 |
| 9 | 29.7 | 15.4 |
| 10 | 28.7 | 15 |

Themaximumrootlengthoftheplantgrownintreatment3(i.e)seedsoaked+fertilizersprayed at10%concentrationofseaweedextractand10%chloroformextractof*Lawsoniainermis*leaf is **36.5cm** and maximum shoot length is found to be**20.2cm**.

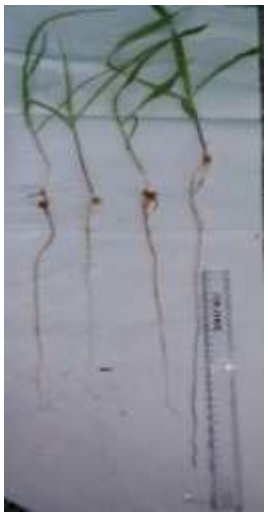


Fig4.11: Representative image of the uprooted plant from the treatment 3 for which the root and shoot length was measured.

**Result:**

**Comparison of root and shoot length of the plants with extract of different concentration:**

The maximum shoot and root length of the plant obtained by treating extract 3 are**36.5cms** and**20**.**2cms**is found to be the tallest plant when compare to the plant grown in different concentration.

**4.5 Over all height of the plantgrown:**

**Extract 1- 1%concentration of seaweedextract and 10% chloroform extract of *Lawsonia inermis* leaf**

**Treatment 1:**

| **Average of shoot and root length of the plant** | | | |
|---|---|---|---|
| **S.No** | **Cms** | **S.No** | **Cms** |
| 1 | 47 | 6 | 44.1 |
| 2 | 46.8 | 7 | 43.8 |
| 3 | 44.2 | 8 | 45 |

| | | | |
|---|---|---|---|
| 4 | 46.6 | 9 | 43.3 |
| 5 | 43.8 | 10 | 43.2 |
| 228.4 | | 219.4 | |
| Average =44.78cm | | | |

**Observation:**

The average total length of the plant grown in treatment 1 (i.e) is **44.78cms**.

**Extract 2- 5% concentration of seaweed extract and 10% chloroform extract of *Lawsoina inermis* leaf**

**Treatment 2:**

| **Average of shoot and root length of the plant** | | | |
|---|---|---|---|
| **S.No** | **Cms** | **S.No** | **Cms** |
| 1 | 51.3 | 6 | 47 |
| 2 | 51 | 7 | 43 |
| 3 | 51.1 | 8 | 45.8 |
| 4 | 48.1 | 9 | 45.1 |
| 5 | 48.2 | 10 | 44.2 |
| 249.7 | | 224.1 | |
| Average = 47.38cm | | | |

The average total length of the plant grown in treatment 2 (i.e) is **47.38cms**.

**Extract3- 10% concentration of seaweed extract and10% chloroform extract of *Lawsoina inermis* leaf:**

**Treatment 3:**

| **Average of shoot and root length of the plant** | | | |
|---|---|---|---|
| **S.No** | **Cms** | **S.No** | **Cms** |
| 1 | 56.7 | 6 | 52 |
| 2 | 56.2 | 7 | 44 |
| 3 | 53.9 | 8 | 47.8 |
| 4 | 54.1 | 9 | 45.1 |
| 5 | 54.2 | 10 | 43.7 |
| 275.1 | | 232.6 | |
| Average = 50.77cm | | | |

The average total length of the plant grown in treatment 3 (i.e.) is **50.77cms**.

**4.6 Total height of theplant**:

Theaveragetotallengthoftheplantgrownintreatment1of1%concentrationofseaweed and 10% chloroform extracts of *Lawsonia inermis* is 44.78cms. And the plant grown in treatment 2 of 5% concentration of seaweed and 10% chloroform extract of *Lawsonia inermis* is 47.38 cm. And the plant grown in treatment 3 of 10%concentration of seaweed and 10%choroform extract of *Lawsonia inermis* is 50.77cm. Therefore ,the plant grown in treatment 3 with 10% concentration of seaweed and 10% chloroform extract of *lawsonia inermis* plant leaf in the condition of seed soaked +fertilizer sprayed is found to be the tallest plant when compared to all the plant grown in different conditions, hence this treatment increases the overall growth of theplant.

## 5. DISCUSSION

The result of present study showed that *Acalypha indica*, *Calotropis gigantea* and *Lawsonia inemis* has antifungal activity against to *Curvularia lunata*. These result corrobate earlier investigation by Somchit et al., (2010), Pawar NT et al., (2018) and Tansukh Barupal et al., (2019).

Antimicrobial activity of *Lawsonia inermis* extracts against fungus *Curvularia lunata* has been assessedandacetoneextractshowedgreatestpercent(70.15%)inhibitionofmyceliagrowthoftarget fungi , Mancozeb and bavistin were used as standards. Minimum Inhibitory Concentration (MIC) andtheacetonefractionof*Lawsoniainermis*hasmaximumantifungalactivityandcanbeusedas a powerfulfungicideagainst*Curvularialunata*intreatingleafspotdiseaseofmaizeTansukhBarupal *et al.*, *(* 2017). In the present study the chloroform extracts of *Acalypha indica*, *Calotropis gigantea* and *Lawsonia inermis* have a greater effect than that of acetone. Chloroform extract of *Lawsonia inermis* shows 75% inhibition of *Curvularia lunata* , fluconazole were used as the standards. It indicates that the active compounds can readily be dissolved or extracted in chloroform when compared to ethanol and petroleum ether.

The leaves of *Acalypha indica* possess major phyto chemical constituents include alkaloids, saponins, terpenoids & steroids, flavonoids, tannins, phenolic compounds Pasumarthi Brahmam*et al.,(*2019) and the *Calotropis gigantean* possess useful biological actions belonging mainly to phenolics, flavonoids, glycosides, alkaloids, cardiac glycosides, phytosterols Madhurima Dutta *et al*., (2014).*Lawsoniai nermis*posses cardio glycosides, tannins, phenol Tansukh Barupal et al., (2019).Thephytochemicalscreeningofthestudyshowedthatthepresenceofflavonoids,alkaloids, tannins, phenol in the leaves extract supports for the antifungalactivity.

Plant Growth Biostimulants Based on Different Methods of Seaweed Extraction with Water We explored two methods for obtaining aqueous extracts: boiling and soaking of Baltic seaweeds (EB and ES, resp.). Algal extracts were characterized in terms of polyphenols, micro- and macroelements,lipidscontent,andantibacterialproperties.Theutilitarianpropertieswereexamined in the germination tests on Lepidium sativum for three extract dilutions (0.5, 2.5, and 10%). It was found that the extracts were similar in micro- and macroelement concentrations. Water was proved tobeagoodsolventtoextractphenoliccompounds.Thealgalextractproducedbysoakingbiomass did not show inhibitory effect on *Escherichia coli* and *Staphylococcus aureus* . Only the boiled extract had an inhibitory activity against *E. coli*. Germination tests revealed a positive influence of the

bioproducts on the cultivated plants. In the group treated with 10% EB, plants were 13%longer than in the control group; the content of elements B, Mo, Zn, and Na in the group treated with 10% ES was higher by 76%, 48%, 31%, and 59% than in the control group, respectively; the content of chlorophyll was 2.5 times higher in 0.5% ES than in the control group. Extracts showed the slight impactonthemorphologyofplantsKatarzynaGodlewskaetal.,(2016).Accordingtotheirresultthe present study shows the average total length of the plant grown in tray A of 1%concentration is **44.78cms**. And the plant grown in tray B of 5%concentration is 47.38cms. And the plant grown in tray C of 5% concentration is 50.77cm. Therefore, the plant grown in tray C with 5% concentration extract in the condition of seed soaked +fertilizer sprayed is found to be the lengthiest plant when compared to all the plants grown in differentconditions.

Seaweed liquid extracts were used in different concentrations in different bioassays. Results showthatextractsobtainedfromtwoseaweeds,*A.nodosum*and*S.muticum*,canbepromissory plantbiofertilizerataconcentrationof25%andhadapositiveeffectonseedgermination,plant development, and production investigated by Silva *etal*., (2019). The result of present study showed that extracts obtained from Seaweed *Sargassum muticum* has maximum effect on maize seed germination and plant development at the concentration of10%.

# 6. SUMMARY

The present study reveals that the that the three plants namely *Acalypha indica, Calotropis gigantea* and *Lawsonia inermis*shows antifungal activity against to the plant fungal pathogen *Curluraia lunata* and the maximum inhibition of *Curvularia lunata* obtained in the Chloroform extractof plants. And the pytochemical result shows the presence of Alkaloids, Flavanoids, tannins, phenol. *Lawsonia inermis* plantsshows inhibition of *Curvulari alunata* in all three solvents (petroleumether,chloroformandmethanol)whencompareto *Acalyphaindica*and*Calotropis gigantae.*

Extract of Seaweed *Sargassum muticum of* differentconcentration1%, 5% and10%respectively shows the germination of maize seed and development of plant. Increase intheconcentrationoftheSeaweed*Sargassummuticum*leadstoincreasetheseedgermination and growth of the maizeplant.

Inconclusion,At10%concentrationoftheSeaweedextractthemaximumgrowthofthemaize plant obtained. The average total length of plant in treatment 3 is 50.77cm which is considered to be a lengthiest plant when compare to the plant in treatment 1 is 44.78cm and treatment 2 is47.38cm.

In future ,GC-MS and molecular docking studies of *Lawsoina inerm*is leaf chloroform extract to be perform to find the specific biological compound responsible for the inhibition of plant fungalpathogen*Curvularialunta*.SothattheactivecompoundfromtheGC-MSandmolecular docking analysis can be tested against the *Curvularia lunta* and can be used with the seaweed *Sargassummuticum*toproducepolyherbalenrichedbiofertilizeragainstplantfungalpathogen. Entirestageoftheplantgrowthrateandtheresponseof*Curvularialunta*towardsplanttreated with poyherbal enriched biofertlizer can bestudied

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