EFFECT OF POTTING MIXTURES ON INVITRO RAISED PLANTLETS OF *Picrorhiza kurroa*

PROJECT REPORT SUBMITTED IN THE PARTIAL FULFILLMENT OF BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

BY

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UNDER THE SUPERVISION OF:

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DECLARATION

We hereby declare that the work presented in this report entitled "**EFFECT OF POTTING MIXTURES ON IN-VITRO RAISED PLANTLETS OF*Picrorhiza kurroa***" in partial fulfillment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted in the Department of Biotechnology & Bioinformatics,Jaypee University of Information Technology Waknaghat is an authentic record of my own work carried out over a period from August 2022 to May 2023 under the supervision of (Dr. Hemant Sood) (Associate Professor, Department of Biotechnology& Bioinformatics). We also authenticate that we have carried out the above mentioned project work under the proficiency stream

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It was really helpful to us, and we learned many interesting facts regarding the growth and development of in-vitro raised plantlets of *Picrorhiza kurroa*. We were also enlightened to various methods of preparing bio-compost using different household items, the different types of potting mixtures using items in varying composition and techniques for breaking down cigarette butts for minimizing its adverse environmental affects.

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LIST OF ABBREVIATIONS

CBs	Cigarette Butts
MS	Murashige and Skoog
IBA	Indole-3-butyric acid
P. kurroa	*Picrorhiza kurroa*
KN	Kinetin
LAF	Laminar Airflow
UV	Ultra violet
HCl	Hydrochloric acid
NaCl	Sodium Chloride
NaOH	Sodium Hydroixde
C	Carbon
N	Nitrogen
CuSO4	Copper Sulphate
K2SO4	Potassium Sulphate
H2SO4	Sulphuric Acid

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ABSTRACT

Being one of the most littered items of the world, cigarette filters pose as perhaps the most toxic waste which pollutes land and oceans to the core all at once. Global efforts for this issue today, are not just a thing to ponder upon but a huge necessity before us. Degradation of such toxic waste in a sustainable yet cheaper ways, such that each country can adopt it and develop into a worldwide phenomenon is the need of the hour. One of the best ways to solve this problem is to treat those CBs with de-acytalation process so that the cellulose acetate could break down and to mix that in the soil or bio-compost for its faster degradation in nature. Cigarette filters contain 95% of cellulose acetate (plastic), a plasticizer, triacetin (glycerol triacetate), is applied to bond the fibers and It contains more than 12,000 white fibers and microscopically these fibers are Y shaped and contain the delustrant titanium dioxide. Our study aims in creating bio-compost with the residual organic household wastes such as wet waste of kitchen, coconut husk powder, vegetable and fruit peels with varying ratios of (5%, 10% and 15%)CBs in it. Then, the survival of medicinal plant *P.kurroa* was checked in all the different potting mixtures after 4 weeks of observation. The result showed that the plant died in the untreated CBs in the bio-compost, survived in the treated ones and the best growth was shown in the vermiculite and perlite potting mixture. The nitrogen content obtained in the different potting mixtures was also obtained and the bio-compost with treated CBs had 0.98% and the N content of Vermiculite perlite was 1.1%. This is the noble experiment which has been carried out in the medicinal endangered plant *P.kurroa* with the purpose of degradation of cigarette filters and eradication of waste altogether. It will be beneficial for the environment and has the potential to improve the pollution.

CHAPTER 1

INTRODUCTION

The Himalayan highlands are home to the plant known as *Picrorhiza kurroa*. The root and rhizome (underground stem) are used for healing by people, particularly by Ayurvedic physicians. *Picrorhiza*is endangered as a result of overharvesting. Jaundice , acute viral hepatitis, fever, allergies, and asthma are all treated with *Picrorhiza.* Additionally vitiligo and eczema are among the skin disorders it is used to treat.It acts as a hepato-protective agent. There is a problem in the regeneration process mainly of the Kuffer cells and it here when extracts of this plant from rhizome and roots of this plant prominently helps in regeneration. The stomach is given support, gastric juices are secreted, and hunger is increased. Additionally, it enhances bowel motionsandaids in the treatment of constipation. It helps in lowering the blood glucose level, serum lipid peroxides and blood urea nitrogen.The presence of substances like kutkin demonstrates the liver's defensive function. It also helps in the treatment of non-alcohol related fatty liver disease. [1, 3, 5]

Bio-compost is manufactured from garbage of homes and from animal wastes, and they are utilized in a variety of agricultural practices. It is a term used to describe matter from plants and animals that has been decomposed and is utilized as fertilizer or manure. It is a crucial element of organic farming. It is packed with nutrients. Bio-composting is the process of decomposing organic matter by simply piling trash in a field or other outdoor area and leaving it there undisturbed for a few days, weeks, or longer. Wet wastes from kitchen includes coconut husk powder, sour buttermilk semi done compost, pine cone, banana peels, potato peels, cucumber peels, onion peels, soil, dry leaves and water content. Bio-compost enhances soil durability and provides the soil with beneficial microbes. In comparison to the soil and organic items that worms eat, worm castings have 10–20 times more micro-organisms. It increases the amount of water that may be stored in the soil by attracting existing, deep-burrowing earthworms. Increases water storage capacity. [2, 8, 9]

Cigarette Filters are constructed of cellulose acetate, a form of plastic that can take up to ten years to degrade, and are the most littered object in the world.The annual production of 6000 billion cigarettes uses plastic filters. It involves over a million tons of plastic. 2/3rds of CBs are haphazardly dumped into rivers and oceans. In certain nations, litterbugs are liable to penalties. Because of the high quantity of acetate substitution, the bacteria have a difficult time breaking down the cellulose biologically. Esterases(enzymes) can be employed to cleave the acetyl group found in cellulose acetate during the initial pre-treatment since cellulose (polymer) can be biodegraded by microbial organisms, but the procedures are very slow (degradation). Enzymes like cellulase can also be used to partially degrade the materials. Photochemical methods like use of UV lights shorter than 280nm can also be used to degrade to some extent. Titanium dioxide addition enhances the photo degradation process. De-acetylation has been proven in studies to dramatically enhance cellulose content from 54.94% to almost 94.61% while decreasing the acetyl group concentration from 46.25% to almost 4.66%. The potting mixtures of Bio-compost and treated CBs were prepared to establish a comparative analysis study with respect to the standardized sample. [7, 8, 19]

Perlite is a silica-based volcanic rock that has been expanded and made less dense by natural gas heating. It enhances the mixing process' ability to hold water and aerate. The pH of perlite is neutral, and it is sterile. When coupled with peat or high C: Ncompost makes up typically30–50% of mixes. Vermiculite is a micaceous material that has been heated with natural gas in a furnace.When handled forcefully, it may lose its ability to hold air. K, Mg, and other trace minerals are some that vermiculite can provide. When coupled with peat or high C:N compost, makes up typically 30–50% of mixes.[22]

Untreated CBs when used in potting mixtures together with bio-compost it was found that paper wrappers of CBs broke down relatively quickly from around the cigarette butt as a result of microbial decay but the filters themselves had only sustained minimal signs of decomposition and microbial attack. The decomposition of discarded cigarette butts was being delayed by the tightly woven fibers within the filter and the plasticizers which are used to fuse the fibers together. The combination of these two factors prohibits the composting bacteria from accessing the filter fibers easily and as such, significantly decelerates their rate of decomposition.

Therefore the present study has been carried out to ascertain the use of CBs in Bio-compost and to create a comparative analysis of their effects on the plants with respect to standardized potting mixtures for the purpose of eradicating the various environmental issues.

CHAPTER 2

REVIEW OF LITERATURE

2.1.Description of *Picrorhizakurroa* [1]

Fig1- *Picrorhiza kurroa*

Leaves: 5–15 cm long, nearly all-base, frequently wilted leaves. The leaves have rough teeth and narrow to a bent stalk.

Rhizomes: Its length is usually between 15-20 cm and it is woody in nature.

Flowers: They are small and purplish blue in color and have cylindrical spikes. The length of the flower is about 8mm, has 5 lobes to the middle and it has comparatively much longer stamens.

Fruits: 1.3 cm long.

Chemistry: The bitter glycoside Kutkin, which contains the C-9 iridoid glycosides Picroside I and Kutakoside, is the core element of its chemical components.

2.2. **Micropropagation of *Picrorhizakurroa***[3, 4, 6]

One of the significant plant species among the different therapeutic plants is the Himalayan indigenous *Picrorhizakurroa*. This significant plant species is being preserved in large part thanks to biotechnology. *P.kurroa* has undergone increasing biotechnological advancement during the past 23 years. In vitro culture techniques have been developed, including plant propagation, synthetic seed synthesis, and micro-propagation.

Perhaps the earliest report on *P.kurroa* tissue culture utilizing shoot tips as explants collected from wild plants kept under controlled circumstances was by Lal et al. (1988). They discovered that kinetin (3.0-5.0 mg/l) supplemented MS medium encouraged the fast multiplication of many shoots from the explants. When IAA (1.0 mg/l) was added to the Kn-containing medium, regenerates grew noticeably better.

For this crucial plant *P. kurroa*, Sood and Chauhan (2009a) have created a low-cost micro-propagation technique. The highest results were obtained from axillary shoot tips that were cultivated on MS media supplemented with 2 mg/l IBA and 3 mg/l Kn, with 86.3% of shoot apices multiplying into multiple shoots. Agar was totally left out, and table sugar was used in its stead. The MS liquid media supplemented with IBA (2 mg/l)Kn (3 mg/l)was the most effective of the six low-cost media combinations examined. With 27 shoots per explants, table sugar 3% (w/v) was shown to be the best.

2.3.POTTING MIXTURES [22, 23 , 24 , 25, 27]

Potting mix is made up of organic ingredients like peat moss and elements that are intended to improve drainage rather than dirt. Sometimes the labels on these bagged goods read "soilless mix" or "soilless medium." Most container gardening, both indoors and outdoors, uses this as the standard product. Slow-release fertilizers are occasionally a part of them.

Properties of Potting mixtures –

Contains no dirt; is soilless.

Since it is sterile and free of disease-causing bacteria, plants can benefit from its safety.

Include elements to enhance aeration and drainage, such as peat moss, sphagnum moss, old bark (pine bark), coir (from coconut husks), pumice, perlite, or vermiculite.

May includes additional fertilizer or slow-release starting fertilizer.

Fluffy and lightweight.

Some Common types of potting mixtures are -

- **Orchid potting mix:** For orchids, all-purpose potting soil contains too much water and too little air. The bark in specialized orchid potting soil helps it drain efficiently and produce the airflow that orchids need.
- **Succulent and cactus potting mix:** Succulents and cacti require quick drainage, which is made possible by the sand in this type of potting soil. Additionally, in order to maintain the pH of the potting mix at a slightly alkaline level, it also incorporates recycled forest products and pH adjusters such oyster shell lime.
- **African violet potting mix:** A specifically designed potting soil that satisfies the pH requirements of 6.0 to 6.5 by adding pH adjusters like dolomitic lime.

- **Organic potting mix:** Everything that goes into making potting mix is certified organic by the OMRI (Organic Materials Review Institute) certification. Only organic materials, such as bone meal or blood meal, are utilized if the mixture incorporates fertilizer.
- **Moisture-control potting mix:**Due to the use of sphagnum moss, coir, and moisture control ingredients such a non-toxic polymer, some potting mixes have the ability to absorb and retain more water than typical potting mixes.

Studies have demonstrated that the combination of soil and farmyard manure (3;1) had the highest sprouting rate, followed by coco peat, leaf manure, and compost (1:1:1).The greatest increases in plant height, number of leaves, root length, and dry weight of roots were observed with coco peat + leaf manure + compost (1:1:2). [5]

Additionally, coco peat + leaf manure + compost (1:1:1) was found to have the highest survival rate, percent increase in girth of graft, number of shoots, leaf area, and relative growth rate. [25]

2.3.1. <u>Vermiculite & Perlite as potting mixture</u> [22]

To improve the structure and qualities of a potting media, perlite and vermiculite are used as soil substrates.

Although they are both put to soil to improve aeration, perlite and vermiculite are significantly distinct from one another in nature. Vermiculite is better for plants since it holds water and nutrients and releases them gradually and controllably, unlike Perlite, which does not hold as much water and provides the soil a more porous quality.

<u>Perlite</u>

Molten rock, commonly known as volcanic glass, or magmatic rock, is the source of perlite. Rapidly heating volcanic glass in industrial furnaces causes the trapped water molecules to convert to steam and inflate the perlite particles. 7.0 to 7.5 is the pH range. The horticultural industry values expanded perlite for being:

• Lightweight for simple blending with other media

• Better aeration

• No organic pollutants

• Pest-proof

Vermiculite

Vermiculite is a reliable growing aid that is frequently utilised in the horticulture sector. It is a mica-like mineral made of hydrated magnesium aluminium sheet silicate. Horticulturists frequently choose it because it attracts nutrients and gently releases them back into the soil, enhancing the growing cycle. 6.5 to 7.0 is the pH range. The advantages of horticultural vermiculite include:

• Better aeration

• Moisture retention

• Faster root growth

• Retention of nutrients including potassium, calcium, and magnesium

• Lightweight for simple mixing with other medium

In recent study different ratios of potting materials like peat moss, vermiculite, and perlite were combined. During the experiment, measurements and records were made of the parameters including survival rate, number of leaves, plant height, and root length.

The percentage of plant survival, root length, plant height, and number of leaves of the tea clone Iran 100 were all raised when the mixture of peat moss, vermiculite, and perlite (2:1:1) was used. [22]

2.3.2. Bio-Compost as potting mixture[2, 8, 26]

- By mixing and piling organic debris to encourage aerobic and/or anaerobic degradation, compost is created through the biological breakdown of organic matter. Pathogens, weed seeds that can germinate, and smells are reduced by composting. While offering necessary nutrients, composts contain water and offer aeration. Compost is not frequently used for organic medium due to the absence of constant, high-quality supply.
- Characteristics- A pH of 6.5-8 was found ideal for compost used in potting mixtures. Commonly, composts had 2% nitrogen, 0.5–1% phosphorus, and

2%potassium. From 16.6 to 23.89% of the total was organic carbon. The results for the total organic matter ranged from 28.60 to 41.20%.

The values of the C/N ratio ranged from 14.22:1 to 18.52:1. Between 420 and 655 kg m-3, the bulk density value was found. The values for the moisture content ranged from 23.50 to 32.10%. 3.50 to 4.40 g water/g dry were the figures for the water retention capacity. **[26]**

In terms of statistics according to studies, crop production in the food residuals compost was comparable to that in the control treatment, which included commercial peat-based potting soil and synthetic fertilizer. Horse-bedding compost utilized at 100% or in a 50%/50 combination with a commercial substrate of bark, peat, and sand resulted in crop growth that was unsuitable for commercial production. [24]

2.3.3. Cigarette Butts with Bio-compost as potting mixture [21, 2, 8,]

According to studies, cigarettes filters are constructed from synthetic plastics and do not degrade. Additionally, cigarette butts produce hazardous compounds that are toxic to both humans and animals when they come into contact with water.

Studies have demonstrated the impact of CBs on the germination and early development of plants. In a study, the toxicity test for CBs was conducted using seeds from Sinapis alba L. and Hordeum vulgare L. It is clear from the data that plants benefit from the low levels of harmful chemicals found in CBs. High CB concentrations in a water solution have been shown to have detrimental impacts on seed germination and radical development. [21]

The toxic substances that are produced from CBs when they are improperly disposed of can hinder plant growth and accumulate in them, making them a significant source of environmental contamination.

Solvent systems like aqueous alkali, were assessed for their abilities to produce high purity and digestible cellulose. Results have shown that after the treatment the acetyl group content in CBs was significantly decreased from 46.65% to 4.66%, corresponding to the cellulose content increase from 54.94% to 94.61 %. [27]

2.4.BIO-COMPOST:

Fig 2- Bio-compost

(https://www.riyaagro.co.in/bio-compost.htm)

Bio-compost is manufactured from garbage of homes and from animal wastes, and they are utilized in a variety of agricultural practices. It is a term used to describe matter from plants and animals that has been decomposed and is utilized as fertilizer or manure. It is a crucial element of organic farming. It is packed with nutrients. Bio-composting is the process of decomposing organic matter by simply piling trash in a field or other outdoor area and leaving it there undisturbed for a few days, weeks, or longer. It is advantageous to use bio-compost in ecosystems to cover landfills, minimize soil erosion, and create wetlands. One of the procedures in the contemporary bio-composting process is the regular tracking of the compost. [2]

Wet wastes from kitchen includescoconut husk powder, sour buttermilk semi done compost, pine cone, banana peels, potato peels, cucumber peels, onion peels, soil, dry leaves and water content. [8]

2.5. **Benefits of bio-compost:** [10, 11]

- Enhances soil durability and provides the soil with beneficial microbes.
- In comparison to the soil and organic items that worms eat, worm castings have 10–20 times more microorganisms.
- Increases the amount of water that may be stored in the soil by attracting existing, deep-burrowing earthworms.
- Increases water storage capacity.

Plant Growth:

Increases root structure and growth; increases crop production; increases germination; and enriches soil with microorganisms.

Economic:

- The reduction of trash flow to landfills as a result of bio-waste conversion.
- The elimination of bio-wastes from the effluents decreases the risk that more recyclables recovered in the same container may becomeinfected.
- Provides employment options for unemployed people in rural areas.
- Because of its low initial cost and straightforward procedures, vermicomposting is practical even in agriculturally underdeveloped areas.

Environmental affects:

- Reduces the overall organic household and commercial wastes from the environment.
- Closes the "metabolic gap" by composting trash locally.
- While mechanical harvesting and temperature control are typically utilized in large systems, other equipment is simple and has a longer life expectancy.
- Production-related emissions of methane and nitric oxide are reduced.

2.6. Ingredients required [8, 12, 7]

- Compost requires three ingredients

  - Browns: includes dead leaves, branches &twigs.(provides carbon for the compost)

  - Greens: includes grass clippings, vegetable waste, fruit scraps (provides nitrogen to the compost)

  - Water: (moisture) to break down the organic matter.

  - Cigarette filters: to use it and reduce its waste from the environment.

2.7. The good and the bad stuff in a bio-compost:[8. 12]

Table 1- The list of different ingredients that are good and bad for the compost.

1.Good stuff	**Foods and vegetables, pine cone, dry leaves, paper, coco peat, saw-dust, tea bags, hay and straw, hair and fur, manure from herbivores, Grass clippings.**
2.Bad stuff	**Pet waste from carnivorous animals, meat and bone scraps, fat oils and grease, plastics and metals, pesticides, colored papers.**

2.8.Properties of the ingredients used: [8 , 9, 13]

-Cocopeat: It is also known as coir. It has great pore space, moisture retaining capacity, very slow biodegradation, low bulk density and renewable. It is light in weight, good source of nutrients and water holding capacity.

-Vegetable peels: It adds vitamins like Vit-E, C, A and various anti-oxidants(onion peel), Iron, calcium potassium and other nutrients.

-Fruit peels: It provides Vitamin – B6, magnesium, carbohydrates, fibers and proteins.

-Pine cone:It contain protein, carbs, fat, vitamin K, vitamin B1 (thiamine), and magnesium.

-Dry leaves- It provides carbon, nitrogen, phosphorus and potassium and improves moisture retention and drainage of excess water.
-CBs- Aromatic and heterocyclic amines, carbonylated compounds, phenols, polycyclic aromatic hydrocarbons, nitrogen oxides and ammonia. [7]

2.9.Report on Micropropagation of *Picrorhiza kurroa*and bio-compost.

Table 2 - Report of some of the authors on *P.kurroa* and bio-compost

Author.	Objectives
H.Sood, R.S Chauhan	Development of a low cost micropropagation technology for an endangered medicinal herb(*Picrorhiza kurroa*)
Yukti, Khushi Maheshwari	Effect of homemade bio-compost and bio-enzymes for growing exotic vegetables.
N.Sharma, H.Sood	In Vitro Production of Medicinal Compounds from Endangered and Commercially Important Medicinal Plants
Thomas E novotny, Kristen Lum et.al	Cigarette butts and the case for an environmental policy on Hazardous cigarette waste.
Tahseen Sayara, Rezq-Basheer Salimia	Recycling of Organic Wastes through Composting: Process Performance and Compost Application in Agriculture

2.10. Components of Cigarette Butts [7, 18 , 19]

Fig 3 – Deconstructed Cigarette Butts

The Cigarette Filter Components include –

Cellulose Acetate Plug

A material called cellulose acetate makes up 95% of cigarette filters; the remaining 5% are made of papers and rayon. It is possible for the cellulose acetate tow fibres to resemble cotton since they are white, less densely packed, and thinner than sewing thread.

An inner paper wrapper and adhesive

For regular cigarettes, the paper used to wrap the acetate cellulose plug is airtight; for "light" cigarettes, the paper is ventilated and very porous, allowing more air to enter the smoke mixture. The glue used to affix the plug to the wrapper and seam the wrapper is a polyvinyl acetate emulsion.

A cover sheet (tipping sheet)

The tipping paper covers the plug on the filter and fastens it to the tobacco column. This paper is frequently patterned to resemble cork. Smokers' lips won't stick to tipping paper because of its special formulation.

As an **additional filtration** agent, some cigarette filters also include charcoal.

2.11. Reports on utilization of Cigarette Butts and its degradation

Table 3- Report of some of the authors on utilization of Cigarette Butts and its degradation

Author.	Objectives
Putri Amanda, Anisyah Putri , Nanang Masruchin , Wida , Riska , Surya , Ismadi	Cellulose was fabricated by de-acetylation of Cigarette butts with NaOH solution at various times 15 , 30 ,45 and 60 minutes in autoclave .
Yamashita , Endo , 2004	The deterioration behaviour of Cellulose acetate films was examines in Hydrochloric acid and Sodium Hydroxide solutions of various concentrations .
Abdullah Iqbal	Chitosan was produced from shrimp waste by deacetylation with 60 % NaOH for 24 hours at 60 degree Celsius.
Najua Tulos , David , Andrew , Parikshit Goswami , Richard	Cellulose Acetate was converted to cellulose II through de-acetylation process using ethanoic NaOH solution.

2.12. Why Pretreatment of Cigarette butts is required [15, 16 ,17]

Most Cigarette Butts are composed of Cellulose Acetate, a form of plastic. Cellulose acetate is prepared by acetylating cellulose, the polymer.

Cellulose is readily biodegraded by organisms that utilize cellulase enzymes but due to additional acetyl group the degradation of cellulose acetate becomes difficult.

2.12.1. Boiling

The three ingredients that make up a cigarette butt are ash, unburned tobacco and filtering material. According to studies, when ash and unburned tobacco are properly disposed of, very little chemical contamination is present in the filtration material. In essence, boiling removes the dangerous compounds that are still lodged in cigarette filters after smoking.

2.12.2. <u>De-acetylation[15, 16,20]</u>

According to studies, untreated CBs do not degrade efficiently or in an environmentally benign way over the short or long term.

Despite this, there are no set protocols or practices for the appropriate management and valuation of CBs in a circular economy perspective. CBs are only land filled and burned in the event that they are recovered.

To speed up the kinetics of its biodegradation, cellulose acetate, which is particularly resistant to biodegradation, must be de-acetylated. To create digestible cellulose, a solvent de-acetylation technique is necessary.

The only one application for CBs was to use the carbs they contained to make levulinic acid, a component used to make bio-fuels and other bio-based products.

To transform cellulose acetate into cellulose, the initial step involved a preliminary de-acetylation stage. Afterward, cellulose was enzymatically hydrolyzed to create fermentable sugars. Finally, the effectiveness of various yeast types in converting them into bio-ethanol was examined. [20]

De-acetylation has been proven in studies to dramatically enhance cellulose content from 54.94% to almost 94.61% while decreasing the acetyl group concentration from 46.25% to almost 4.66%.

According to studies, the enrichment of cellulose and improvement of enzymatic accessibility were caused by the dual function of acetyl group removal and cellulose deconstruction. [15, 16]

60% NaOH solution for 24 hours at 60 degrees Celsius was found to be best for the de-acetylation of Cigarette Butts.

CHAPTER 3

RATIONALE AND OBJECTIVE

3.1. Rationale of the project

- No reports available so far on utilization of CBs for making potting mixtures for growing *Picrorhiza kurroa*
- To reduce one of the most littered wastes in the world (cigarette butts).
- To promote the usage of "sustainable growing media" for growing exotic and rare plants in favourable conditions.

3.2. Objectives

- To carry out the preparation of sustainable growing media
- To carry out the hardening of tissue cultured plants
- To see the effect of potting mixtures on growth and development of *Picrorhiza kurroa*.

CHAPTER 4

METHODS AND MATERIALS

4.1.Micropropagation of *Picrorhizakurroa*

Preparation of Media (IBA Kinetin media in the ratio of 3:1) (1000ml)

- First, the stock solutions from Stock A to Stock H were added in a beaker in different quantities.
- IBA- 3ml and Kinetin- 1ml was added with the stock solutions.
- Then, 30 gm sucrose was added to the solution.
- Took the volume up to 900ml by adding distilled water and checked the pH of the solution, pH must range between 5.6-5.7.
- HCl and Nacl were added to maintain the pH.
- Make up the solution to 1000 ml with distilled water and add 9 gm agar in it.
- Boiled the solution for 15 minutes.
- Transferred the small amounts of solution in the beakers and autoclaved it.
- After autoclaving the mediawas kept at room temperature for 3-4 days to check for contamination.
- The contaminated media were discarded and the uncontaminated media were used in the culturing of the plant.

*4.2.*Sub-culturing of *PicrorhizaKurroa*

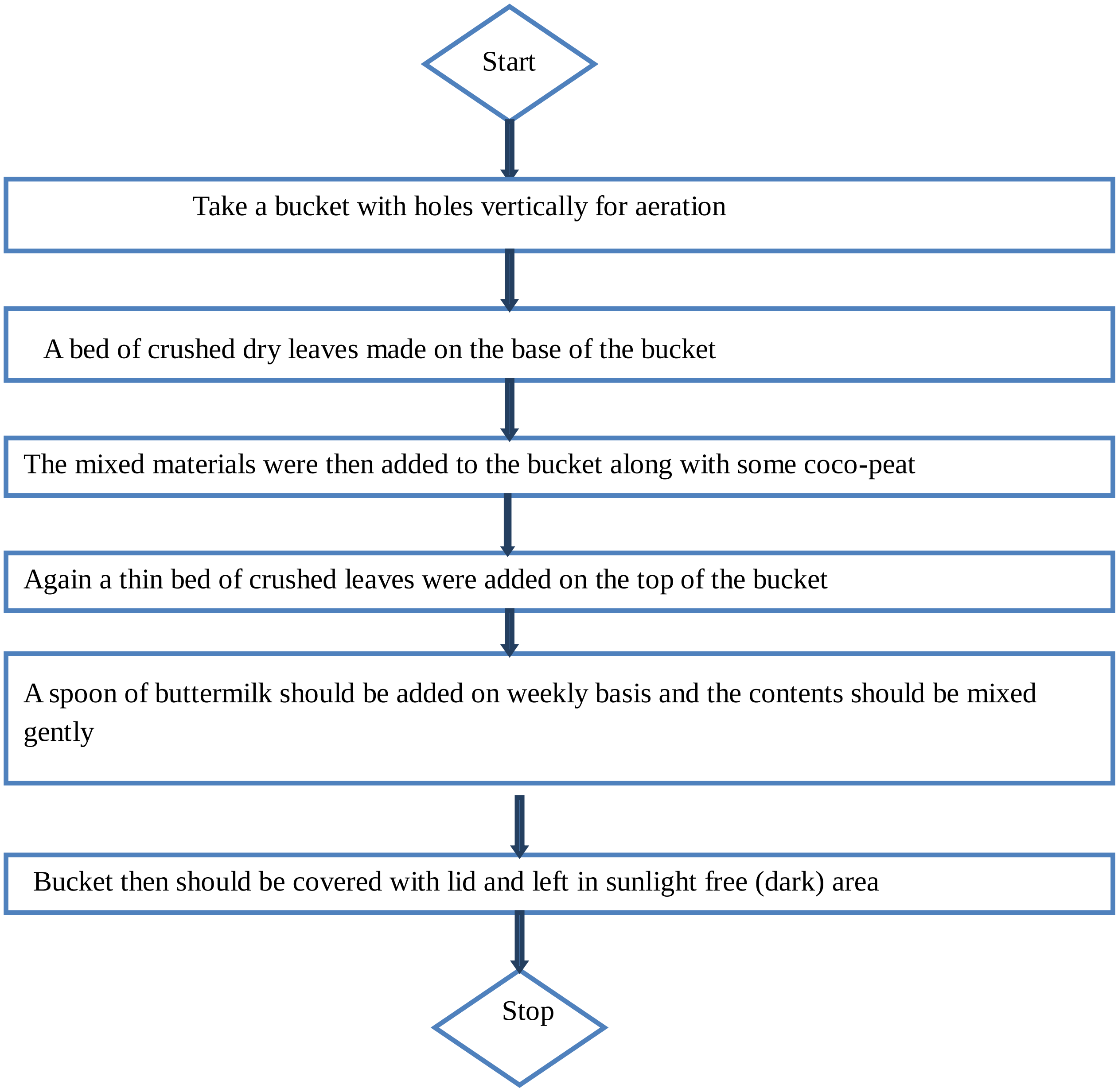
Fig 4- Media jars with explants of *Picrorhizakurroa*

- Placed the objects (Petri plates, scalpel, forceps, media jars, surface sterilizers) required in the procedure in the Laminar Airflow.
- Switched on the UV light of the LAF 15-20 min before starting thework.
- Turned off the UV light of the LAF and transferred the motherplants into the LAF
- Sterilize the culture area: Clean the working area using 70% isopropyl alcohol or ethanol.
- Use 70% isopropyl alcohol or ethanol to sterilize your hands.
- Then, using sterilized forceps and scalpel gently pull the mother plant from the media and split the plant into smaller explants.
- In between the splitting of plants, sterilize your forceps for 10-15 seconds
- Then the explants were transferred into new media jars with the help of forceps
- Then the jars were labeled and moved to the culture room.

4.3. Procedure / Flowchart for preparation of bio compost

For the preparation of the bio-compost different ingredients like raw household wastes when mixed with coco peat, cone pines, paper wastes, dry plant wastes and water were mixed and placed on top of the base / bed of dry leaves in a bucket with proper aeration system.

Table 4– The stepwise methodology for the preparation of bio-compost



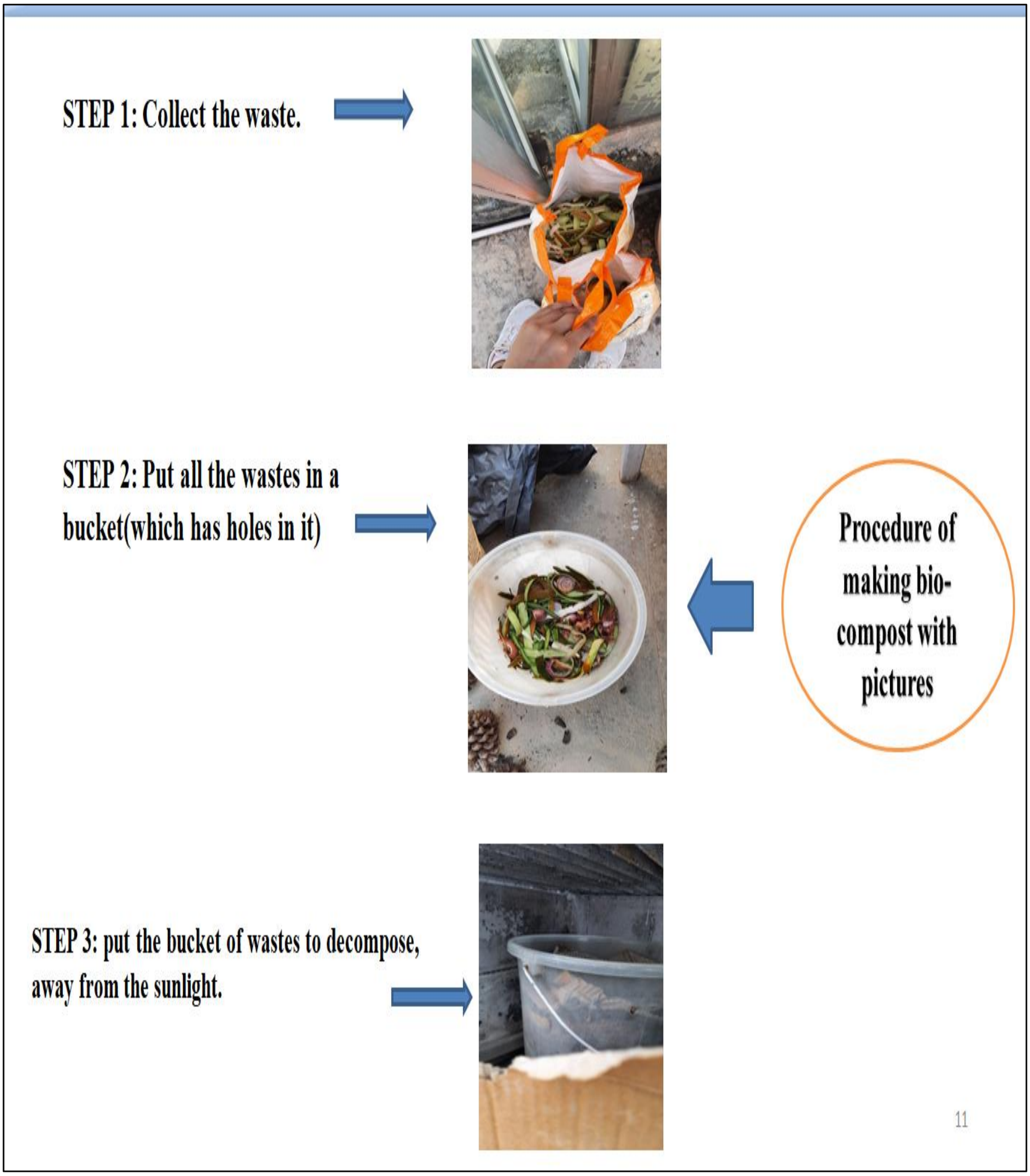


Fig 5- Stepwise Pictorial description of preparation of bio-compost

For the composting process to complete the mixed ingredients (bucket) were masked by a top layer of dry leaves and then the bucket was covered with a lid and kept in a dark (sunlight-free) area for the mandatory amount of time.

4.4. Pretreatment of Cigarette Butts

4.4.1. Boiling of Cigarette Butts

200 gm of Cigarette butts were taken and filled with water in a 500 ml flask until the butts were completely immersed.

Then the flask is put into a water-bath for boiling at 100 degrees Celsius.

The sample is boiled for a time duration of 1 hour.

After an hour of boiling the Cigarette butts are put into greenhouse conditions for the purpose of drying (Overnight).

Later after dying the boiled Cigarette butts are put into the bio-compost for testing purpose.

Fig 6 – Sample obtained after boiling

4.4.2. De-acetylation of Cigarette Butts using Sodium Hydroxide

Prepare a 60 percent NaoH solution for the treatment of Cigarette Butts.

Fig 7- CBs dipped in NaOH solution for the de-acetylation process.

20 gm of Cigarette Butts was weighed.

60 gm of Sodium hydroxide was weighed.

Then placed in a flask and filled with distilled water to make a 100 ml solution

Note – The Cigarette butts needs be completely immersedin the NaOH solution.

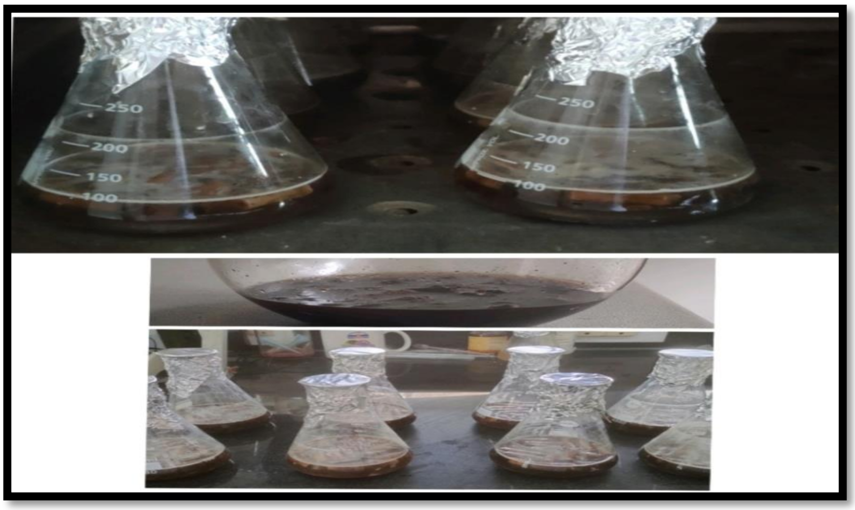


Fig 8 - Samples placed for de-acetylation

16 similar samples were prepared and then placed in a Oven at 60 degree Celsius for Overnight heating (20 hours)

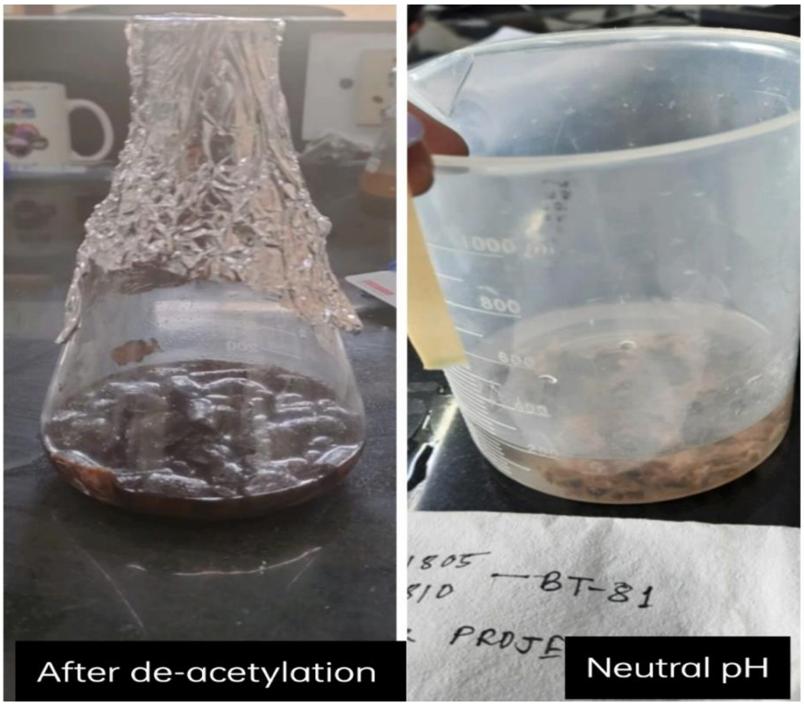


Fig 9 - Samples obtained after 20 hours of de-acetylation

Then the samples were washed and until the pH of the samples become neutral.

8 of the samples were directly put in the bio-compost for testing purpose

The other 8 samples were first dried (oven dried for 6/7 hours accordingly at 100 degrees Celsius)

> **Note** – Dry till the samples turn a bit into powdery form and observe thesamples thoroughly so that it is not over dried / burnt

> **Note** – Here the number of samples prepared (60 % NaOH) were according to the replicates to be prepared.

Fig10- Dried Samples obtained after 7 hours of oven drying

After drying the samples were put in the bio-compost for testing purpose.

4.5.<u>Preparation of different potting mixtures and hardening of plants</u>

In 45 days, the compost was prepared.

Then, different potting mixtures were prepared containing different ratio of CBs and bio-compost.

The amounts were measured with the help of a weighing scale.

Fig 11 - Weighing of CBs using weighing scale

Firstly,standardized samples with no CBs and 200 g vermiculite & perlite were prepared.

Secondly samples containing 200 g of the developed bio-compost were prepared.

Fig 12 -The prepared potting mixtures.

Then samples containing 20 g of untreated Cigarette Butts and 180 g of bio-compost were prepared.

Similarly,samples containing 40 g of untreated Cigarette Butts and 160 g of bio-compost were prepared.

And samples containing 60 g of untreated Cigarette Butts and 140g of bio-compost were prepared.

The next samples were prepared containing 80 g of pretreated Cigarette butts (wet) and 120 g of bio-compost.

Then samples were prepared containing 40 g of boiled Cigarette butts and 160 g of bio-compost.

At last samples were prepared containing 20 g of dried pretreated Cigarette butts (powdery) and 180 g of bio-compost.

Table 5– Composition of the prepared potting mixtures.

Potting mixture	**Composition**
A	200 g vermiculite & perlite (Standardized)
B	200 g of developed Bio-compost
C	20 g of untreated Cigarette Butts + 180 g of bio-compost
D	40 g of untreated Cigarette Butts + 160 g of bio-compost
E	60 g of untreated Cigarette Butts + 140 g of bio-compost
F	80 g of pretreated Cigarette butts (wet) + 120 g of bio-compost.
G	40 g of pretreated (boiled) Cigarette + 160 g of bio-compost.
H	20 g of dried pretreated Cigarette butts (powdery) + 180 g of bio-compost.

4.6. <u>Hardening of the Sub-cultured plants in the prepared potting mixture</u>

Once the previously sub-cultured plants have grown to a decent height and were ready to be hardened in potting mixtures, they were gently extracted from the growth media jars and the roots were washed thoroughly.

For sterilization, both the plants and the potting mixtures were rinsed with fungicide solution (Bavistin (0.1%) and Mercury Chloride (0.5%).

Then the plants were potted in the different potting mixtures prepared and at initial days the pots were covered with glass jars and were kept at greenhouse conditions.

The pots were uncovered (glass jars) for 1-2 hours every day.

The pots later (after 2 weeks) were transferred to normal room conditions for further growth.

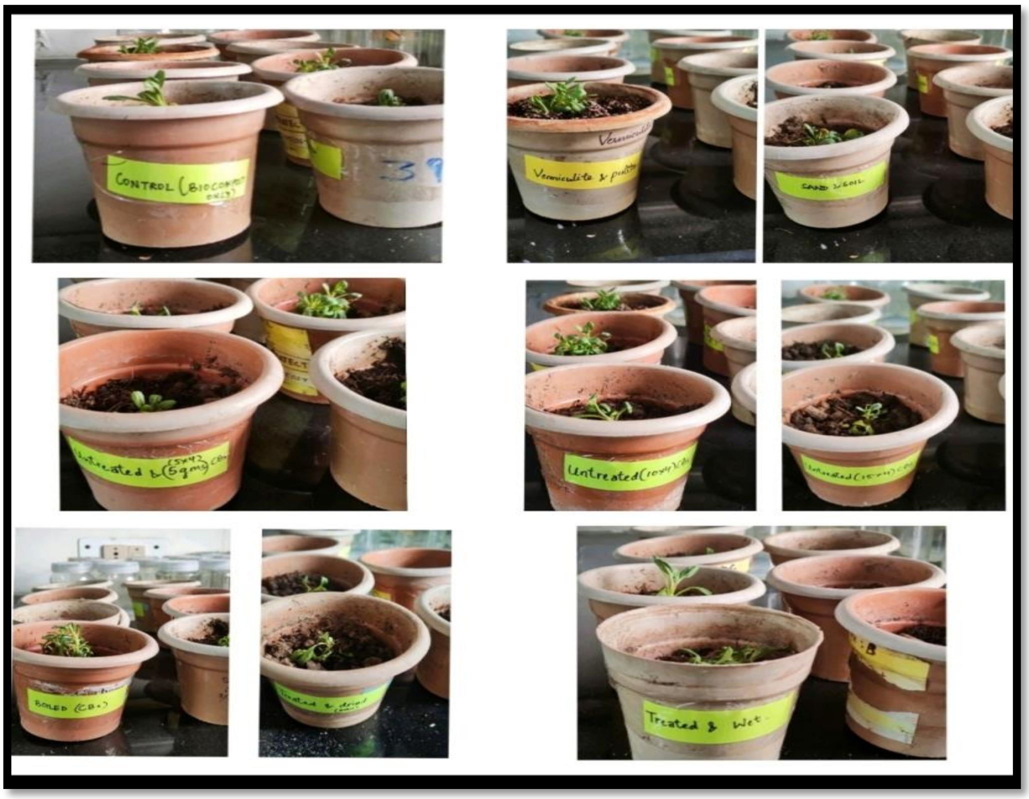


Fig 13– Experimental plants potted in different potting mixtures

4.7. Estimation of Nitrogen/Protein concentration in different potting mixtures.

This process was carried out using the KjelTRON NITROGEN / PROTEIN ESTIMATION STSTEM

4.7.1. Chemicals required ---

- For Digestion-

Conc. Sulphuric Acid : 10ml.

Catalyst Mixture (1:5) : ($CuSo_4$ + K_2So_4) (3gms)

Sample weight : 1 gm

Digestion Time : approx 45mins to 1hr

Temperature : Initial 250C for to avoid frothing than increase the Temperature gradually to 420C

- For Distillation

40 % NaOH : 40-45 ml per sample

4% Boric acid : 25ml per sample

Indicator : 5 drops (Methyl red)

- For Titration

0.02N H_2So_4

4.7.2. Procedure ---

- For Digestion:

The digestion system was preheated to 300 C.

Into the digestion tube, 1g of the sample was placed.

After adding 3 gm of the catalyst mixture, 10 ml of the concentrated H2SO4 was added. Then the tubes along with the manifolds are loaded in the digestion system.

Fig 14 – KjelTRON digestion system

Verify that the manifolds are correctly installed.

Then the scrubber system was turned on immediately.

Check for any sample foaming; if none exists and the sample behavior is normal, we need to raise the temperature to 420C.

After one hour, check to see if the color of the sample has changed to a bluish green before removing the tubes and placing them on the cooling stand.

- <u>For Distillation:</u>

Turn on the primary AC power.

Turn the system switch on.

Keep the tap for distilled water turned on.

Turn on the control panel's power switch.

Take the digested sample in the digestion tube.

Fig 15- KjelTRON system for distillation

Utilizing the slider mechanism, insert the tube into the distillation apparatus.

Take 25ml of 4% boric acid and place it in the receiver end of a 250ml conical flask.

Use the Alkali Button to add 40% alkali.

Watch for the Ready signal to glow. If Ready is glowing, begin distilling the sample.

Check for condensation by opening the tap.

Start distilling the sample by pressing the Steam injection Button.

Take the ammonia collection for titration after distillation, and then shut off the water supply.

- <u>For Titration</u>

  Following titration with 0.02N H_2So_4, the obtained distillate solution changes color to pink.

- <u>Calculation:</u>

  % of Nitrogen = (14 * Normality of Acid * Actual titrant value * 100) / Sample Wt * 1000

Note- The above formula was used to calculate the percentage nitrogen content in the sample tested.

CHAPTER 5

RESULT

5.1. Shoot proliferation of *Picrorhizakurroa*

- MS media prepared with IBA & Kn in the ratio of 3:1 was used. In the LAF using sterilized instruments the mother plant was pulled from the media, split into smaller explants and then gently transferred into new media jars for the shoot proliferation process (as shown in figure 16)

Fig 16- Media jars containing explants of *Picrorhizakurroa*

- After 4 weeks of incubation (as shown in fig 17) 10-12 shoots were grown in each culture flask which would be utilized for further cultivation.

Fig 17 – Fully grown explants of *Picrorhiza kurroa*

5.2. Hardening of the grown explants

After the explants have proliferated their shoots and roots in the media jars and were ready for the hardening process, the plants were gently pulled from the media jars, their roots were washed thorougly and were planted in the pots containing soil and other necessary components and covered with glass jars (as shown in fig 18) for high humidity for the hardening process to complete in greenhouse conditions.

Fig 18- Grown *Picrorhizakurroa* explants planted for hardening

5.3. The prepared bio-compost

After 40-45 days of the composting process satisfactory quality of bio-compost (as shown in figure 19) of dark tan brown color, crumbly, having earthly smell, holding good quantity of moisture, proper pH was obtained.

Fig 19– Bio-compost prepared after decomposition for 45 days

5.4. Change in Characteristics of Cigarette Butts after pre-treatment

5.4.1. After Boiling

As shown in (Figure 6) not much physical change was observed in the fibrous structure of the cigarette butts. Fading of color was observed due to removal of the tar, ash, and other trapped chemicals of the cigarette butts.

5.4.2. After de-acetylation

As shown in (Figure 10)notable changes were observed in the fibrous structure of the Cigarette butts. The fibrous structure was seen to be broken down into simpler thread like structure. The cigarette filters dissolved in the solution to certain extent and the rigid polymeric structure no longer existed, that is the plasticized structure turned into soft cotton like structure.

De-acetylation did not affect the outer paper covering. It is not a matter of concern as it degrades very easily under normal conditions. We can either filter out the paper or use it together with treated CBs when used in Bio-compost.

5.5. Nitrogen percentage obtained in the potting mixtures-

Results have been obtained using the formula –

% of Nitrogen = (14 * Normality of Acid * Actual titrant value * 100) / (Sample Wt * 1000)

Table 6 – Nitrogen percentage of different potting mixtures

Sample no	Composition	Nitrogen percentage
A	Vermiculite & perlite& cocopeat(standardized)	1.1 %
B	Prepared Bio-compost	1.3 %
C	Bio-compost + Untreated CBs	0.80 %
D	Bio-compost + treated (boiled)	0.42 %
E	Bio-compost + treated (de-acetylated & wet)	0.90 %
F	Bio-compost + treated (de-acetylated & dried)	0.98

5.6. Graphical representation of Nitrogen percentage in Different potting mixtures

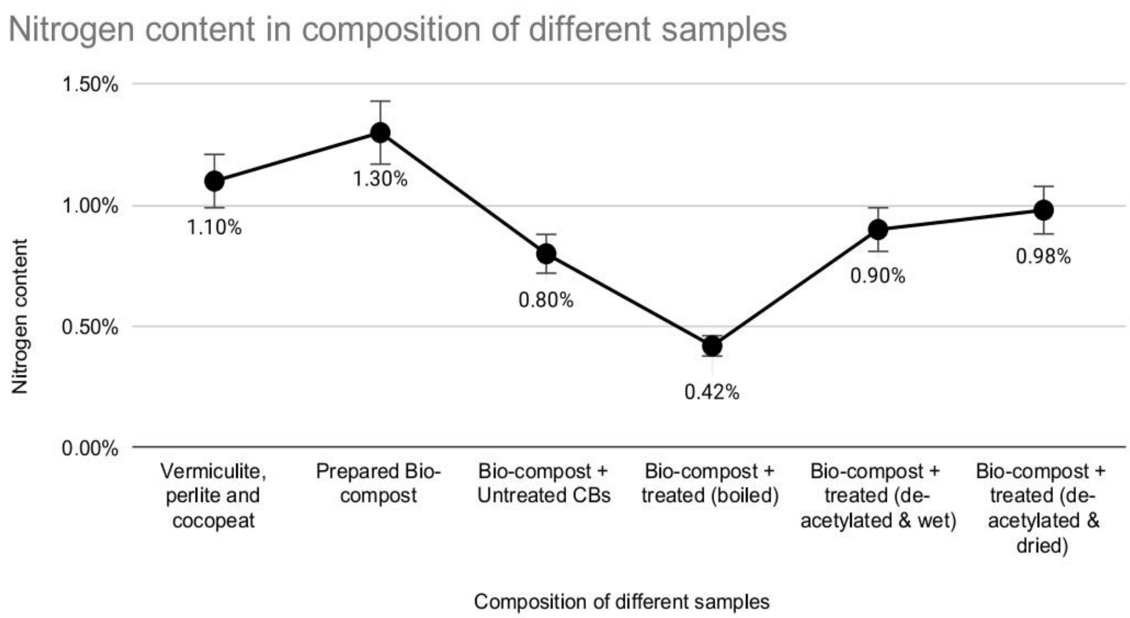


Fig 20 – Nitrogen Content in different potting mixtures

5.7. Comparative analysis of plant growth in different potting mixtures (Vermiculite& perlite(standardized),Developed Bio-compost, Bio-compost containing Cigarette Butts)

5.7.1. Vermiculite & Perlite (standardized)

Experimental plants grown in potting mixture of Vermiculite and perlite (as shown in figure 21) showed decent growth with increase in average height to 7.4 cm from 5.5 cm. Increase in average weight from 0.93 g to 1.52 g was observed and the number of shoots increased from 3 to 6. (Table 7)

Fig 21-Experimental plants grown in potting mixtures of Vermiculite and perlite

5.7.2. Prepared Bio-compost

Experimental plants grown in the developed bio-compost (as shown in figure 22) showed good increase in average height to 7.4 cm from 5.5 cm. Increase in average weight from 0.82 g to 1.09 g was observed and the number of shoots increased from 2 to 4. (Table 8)

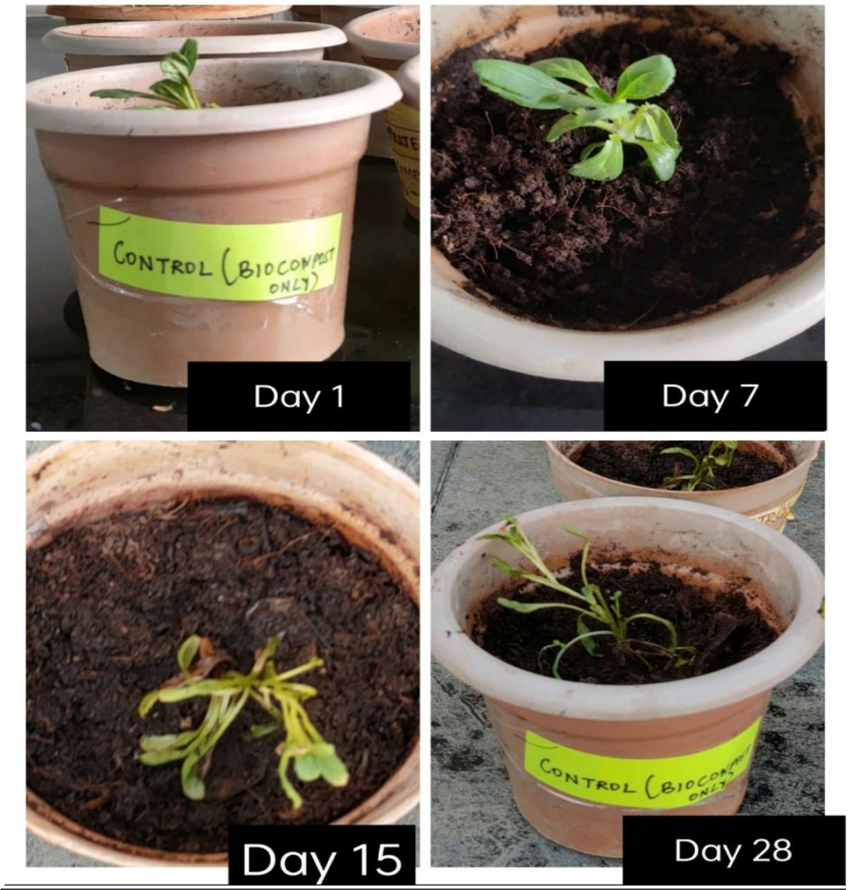


Fig 22-- Experimental plants grown in the developed Bio-compost

5.7.3. Bio-compost & Untreated CBs (20 g)

In experimental plants grown in bio-compost and untreated CBs (20g) (as shown in figure 23) slightly less growth rate was observed in comparison to the standardized samples. Though average height of the plant increased from 4.5 cm to 5.7 cm. Increase in average weight from 0.38 g to 0.84 g was observed and the number of shoots increased from 2 to 3 (Table 9)

Fig 23-Experimental plants grown in potting mixture of Bio-compost and Untreated CBs

5.7.4. Bio-compost & Untreated CBs (40 g & 60 g)

Experimental plants grown in potting mixture of Bio-compost & Untreated CBs (as shown in figure 24) could survive only for around 2 weeks (14-16 days).

For potting mixturescontaining 40 g of CBs by the end of two weeks (until the plant died) the average plant height decreased from 6.5 cm to 3.2 cm. Average weight decreased from 0.93 g to 0.32 g and the number of shoots decreased from 4 to 1. (Table 10)

For potting mixtures containing 60 g of CBs by the end of two weeks (until the plant died) the average plant height decreased from 6.1 cm to 3 cm. Average weight decreased from 0.65 g to 0.37 g and the number of shoots decreased from 3 to 1. (Table 10)

Fig 24-Experimental plants grown in potting mixtures of Bio-compost and Untreated CBs (40 g & 60 g)

5.7.5. Bio-compost & treated (only boiled) CBs

Experimental plants grown in potting mixture of Bio-compost & Boiled CBs (as shown in figure 25) could survive only for around 2 weeks (14-16 days. At the end of two weeks (till the plant survived) the average plant height decreased from 7.5 cm to 3 cm. Average weight decreased from 1.45 g to 0.5 g and the number of shoots decreased from 3 to 1. (Table 11)

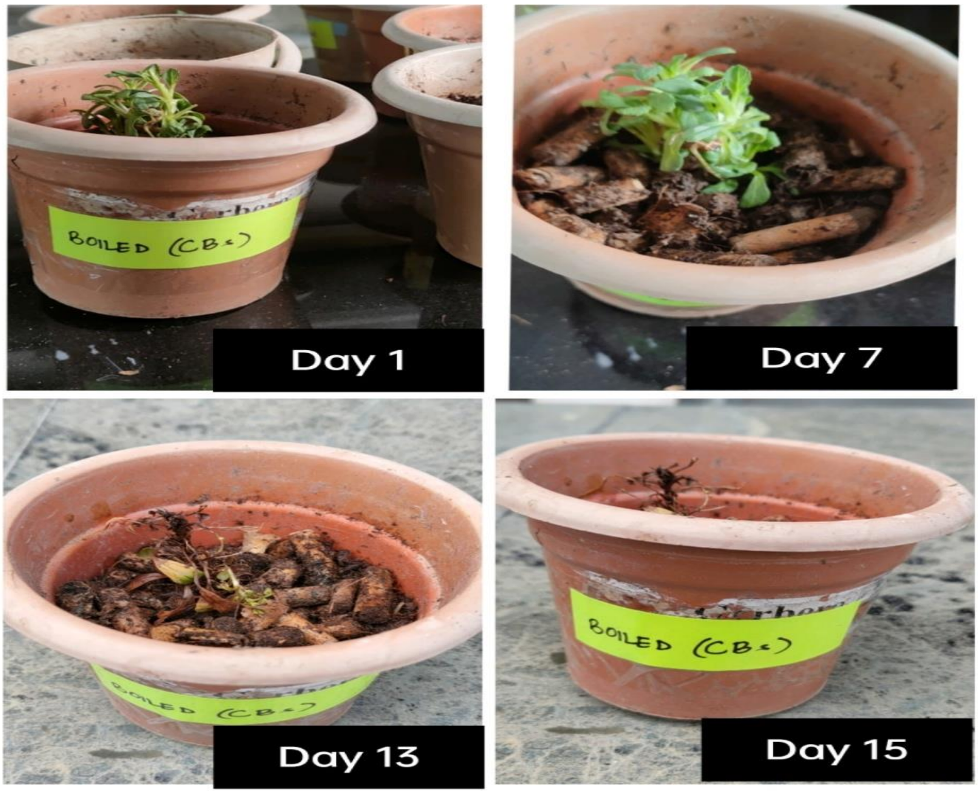


Fig 25-Experimental plants grown in potting mixtures of Bio-compost and treated (Boiled) CBs

5.7.6. Bio-compost &treated CBs (wet)

Experimental plants grown in potting mixture of Bio-compost and treated CBs (wet) (as shown in figure 26) showed decent growth with increase in average height to 6.5 cm from 4.8 cm. Increase in average weight from 0.51 g to 0.91 g was observed and the number of shoots increased from 1 to 3 (Table 12)

Fig 26-Experimental plants grown in potting mixtures of Bio-compost and treated (de-acetylated & wet) Cigarette Butts .

5.7.7. Bio-compost & treated CBs (dried)

Experimental plants grown in potting mixture of Bio-compost and treated CBs (dried) (as shown in figure 27) showed good increase in average height to 4.4 cm from 6.2 cm. Increase in average weight from 0.85 g to 1.05 g was observed and the number of shoots increased from 2 to 4. (Table 13)

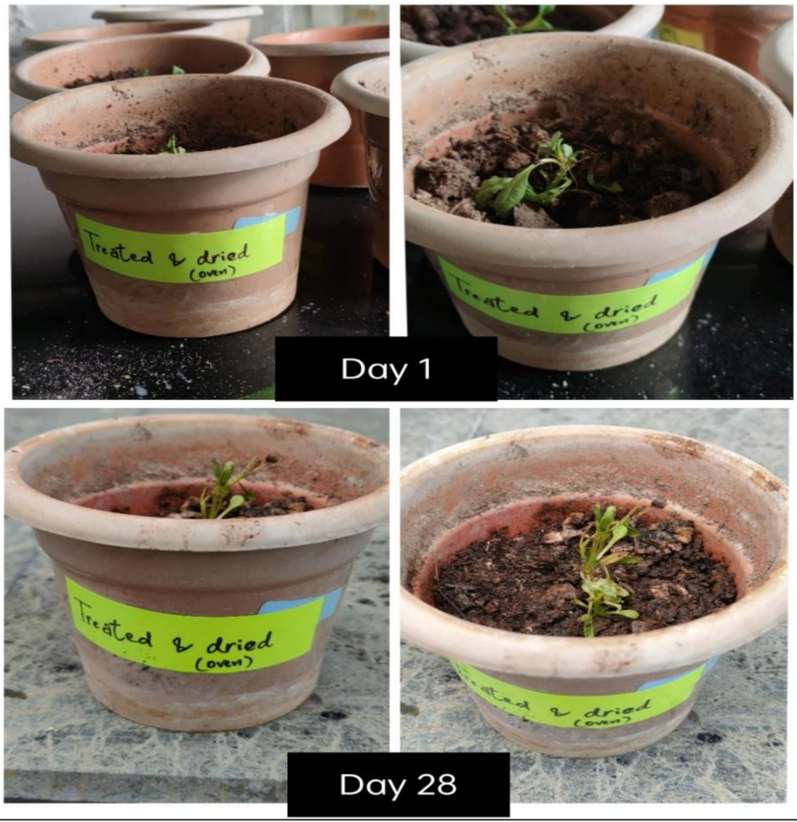


Fig 27-Experimental plants grown in potting mixtures of Bio-compost and treated (de-acetylated & dried) Cigarette Butts .

5.8. Tables for Comparative growth analysis of plants

Table 7: Plants potted in Vermiculite & perlite (standardized)

	Initial	Final (after 4 weeks)
Avg. Height (cm)	5.5 cm	7.4 cm
Avg. Weight (gm)	0.93 g	1.52 gm
Avg. no of shoots	3	6

Table 8: Plants potted in Developed Bio-compost

	Initial	Final (after 4 weeks)
Avg. Height	5.6 cm	7.0 cm
Avg. Weight	0.82 g	1.09 g
Avg. no of shoots	2	4

Table 9: Plants potted in potting mixture containing Untreated CBs (20 g)

	Initial	Final (after 4 weeks)
Avg. Height	4.5 cm	5.7 cm
Avg. Weight	0.38 g	0.84 g
Avg. no of shoots	2	3

Table 10: Plants potted in potting mixture containing Untreated CBs (40 g) & (60 g)

	Initial (40 g CBs)	Final (after 14 days)	Initial (60 g CBs)	Final (after 14 days)
Avg. Height	**6.5 cm**	**3.2 cm**	**6.1 cm**	**3 cm**
Avg. Weight	**0.93 g**	**0.32 g**	**0.65 g**	**0.37 g**
Avg. no of shoots	**4**	**1**	**3**	**1**

Note – The above data was obtained after two weeks of observation

Table 11: Plants potted in potting mixture containing treated (Boiled) CBs

	Initial	Final (after 15 days)
Avg. Height	**7.5**	**3**
Avg. Weight	**1.45 g**	**0.5 g**
Avg. no of shoots	**3**	**1**

Note-The above data was obtained after two weeks of observation

Table 12: Plants potted in potting mixture containing Bio-compost & treated (wet) CBs

	Initial	Final (after 4 weeks)
Avg. Height	**4.8 cm**	**6.5 cm**
Avg. Weight	**0.51 g**	**0.91 g**
Avg. no of shoots	**1**	**3**

Table 13: Plants potted in potting mixture containing Bio-compost &treated (dried) CBs

	Initial	Final (after 4 weeks)
Avg. Height	**4.4 cm**	**6.2 cm**
Avg. Weight	**0.85 g**	**1.05 g**
Avg. no of shoots	**2**	**4**

5.9. Graphical representation of growth pattern of *P.kurroa*

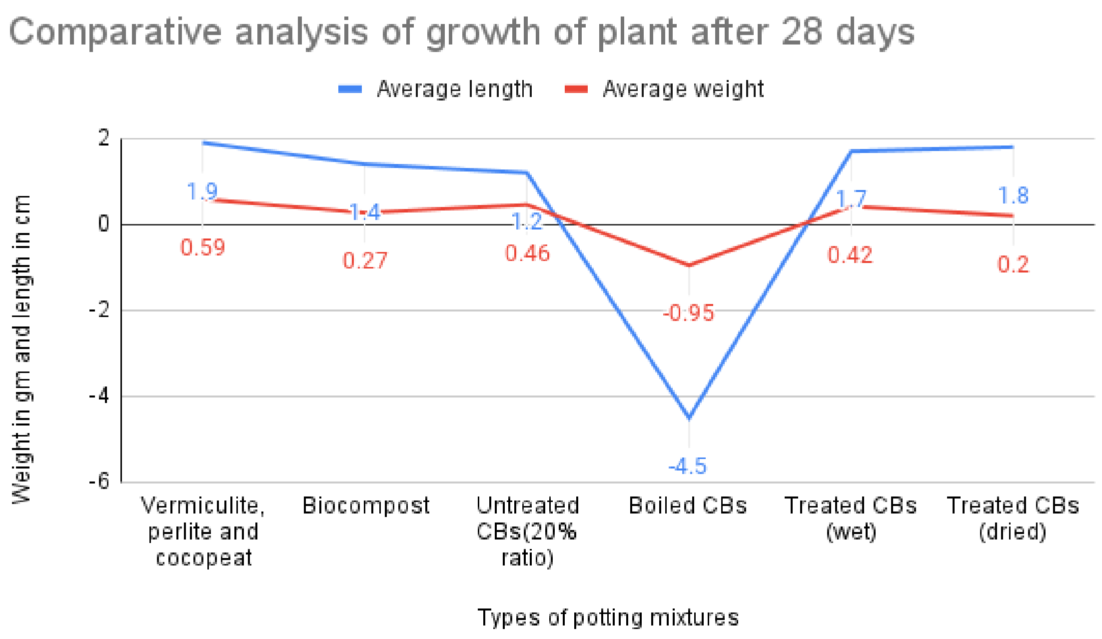


Fig 28- The comparative analysis of growth and development of plant after 28 days.

CHAPTER 6

DISCUSSION

The effect of different potting mixtures on the growth and development of P.kurroa was observed. After analysis, it was found that with respect to the standardized sample(which is vermiculite, perlite and cocopeat 1:1:1) the growth monitored in the developed bio-compost showed good increment in the height, weight, number of leaves and shoots of the plant. In the other potting mixture, which was comprised of Bio-compost and untreated cigarette filters with ration of 20g, 40g and 60g CBs in them respectively. A slightly slower growth rate was observed in 20gm and in samples containing 40g and 60g of CBs, the plant could survive for 14 to 16 days only.

The combination of bio-compost and treated cigarette filters (boiled at 100 degrees) showed detrimental growth and couldn't survive more than 2 weeks. As shown in figure 26, the cultured plants grown in the potting mixture of Bio-compost and treated(de-acetylated) showed decent amount of growth in height, weight and number of shoots.

The nitrogen content was also calculated in the various above mentioned potting mixtures and it was observed that the prepared bio-compost with no cigarette filters showed the highest percentage followed by the vermiculite, perlite and cocopeat combination, Bio-compost and treated(deacetylated), untreated and boiled.

The ratio of cigarette filters in the potting mixtures can determine and control the survival of plants. By boiling the cigarette filters it showed negligible effect on the degradation process. Whereas, when the ratio of CBs is less and it is deacetylated it showed the faster degradation process and we also obtained the good amount of nitrogen content when compared to the standard value.

CHAPTER 7

CONCLUSION AND FUTURE PROSPECTS

From the above studies and experimentation, we can conclude that raw household wastes when mixed with coco peat, cone pines, paper wastes, dry plant wastes and water when added in proper proportion and kept under required environmental conditions can form satisfactory quality of bio-compost holding good quantity of moisture and proper pH.

Supplementation of bio-compost with CBs was carried out in order to utilize it for composting and for protecting the environment from pollution. The prepared bio-compost with CBs would be utilized for hardening the tissue culture plants and its effect on growth and development will be monitored in the coming times.

Cigarette filters are the most toxic littered item which is generally ignored due to its small size. About 6 trillion cigarettes are manufactured a year and over 90% of them contain plastic filters. That's more than one million tonnes of plastic. About two-thirds of butts are dumped irresponsibly and goes straight into the rivers and oceans. Whereas, household and agricultural wastes are also huge threat and it contributes greatly in global warming. These problems could be tackled by making bio-compost and treating the CBs altogether to focus on the sustainability of the environment. This research paper can solve a lot of environmental problems like faster degradation of CBs, usage of agricultural and household wastes etc. In this paper we also came to know that if untreated CBs are kept in the bio-compost for period of 6 months and more, it showed some level of degradation which was a little slow than compared to the treated ones with the Nitrogen content of 0.80%. It can be proved to be one of the easiest ways to get rid of the most littered items of the world.It is the noble work when the *P.kurroa*, the medicinal endangered plant is used in these type of potting mixtures. We can also test these various potting mixtures for commercial plants and determine their growth pattern as well.

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