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Assessment of Mycroflora Effect Found in Soil

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Abstract: *The present study was conducted to isolate and identify fungus present in the soil and to study the effect of the potassiumferrocyanide against them. For this purpose we collected the soil sample from the two different farms which are located near the virar after the collection of the sample the suspension was prepared and they were cultivated on the sterile nutrient agar medium and sterilepotato dextrose agar medium after their growth with the help of standard reference the different types of fungus were identified they were mainly Alternariaalternata, Aspergillusfumigatus, Aspergillusflavus, Candida albicans, Lichtheimiahyalospora, Candida tropicalis, Rhizopusoryzae, Aspergillusterreus. After the identification of the fungus we proceeded to our further experiment which was to check the growth of the fungus on the potassium ferrocyanide. For this purpose we prepared the different types of medium with the use of the chemical and agar medium the chemical which were used were Potassium ferrocynide and agar medium whichever used were sterile nutrient agar and sterilepotato dextrose agar. We prepared two mediums for each chemicals one was of sterile nutrient agar and other was of sterilepotato dextrose agar total 16 plates of potassium ferrocyanide were prepared 8 was on sterile nutrient agar plus chemical and other was of sterilepotato dextrose agar plus chemicals and after the preparation the inoculation of fungus were done on the medium containing the chemical and they were incubated at 37°C fir 48 hour . After the incubation period the growth of the fungus were observed and the resistance and the sensitivity of the fungus against potassium ferrocyanide was studied. The results which were observed were that various fungus were resistant to the potassium ferrocyanide. The species of fungus which were resistant against the potassium ferrocyanide were Aspergillusfumigatus,Aspergillusflavus, Lichtheimiahyalospora, Rhizopusoryzae.*

Keywords: *Alternariaalternata, Aspergillusfumigatus, Aspergillusflavus, Candida albicans, Lichtheimiahyalospora, Candida tropicalis,Rhizopusoryzae, Aspergillusterreus,* resistance and sensitivity

I. INTRODUCTION

Potassium ferrocyanide is the inorganic compound with formula $K_4[Fe(CN)^6]\cdot 3H_2O$. It is the potassium salt of the coordination complex $[Fe(CN)_6]^{4-}$. This salt forms lemon-yellow monoclinic crystals. It's IUPAC name is Potassium hexacyanidoferrate(II).[1] Numerous specialized uses for potassium ferrocyanide are found in industry. It is frequently used as anticaking agents for both table salt and road salt, along with the related sodium salt. The purification of tin and the separation of copper from molybdenum ores are two more processes in which potassium and sodium ferrocyanides are employed. Citric acid and wine are produced using potassium ferrocyanide. It can

ISSN (Online) 2582-9327
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Volume 3, Issue 12, December 2022

be utilized in animal feed as well. As of 2017, ferrocyanides (E 535-538) were only permitted in two food categories as salt additives in the EU. [2] The kidneys are the organ most affected by ferrocyanide toxicity. In the lab, potassium ferrocyanide is used to determine the concentration of potassium permanganate, a compound commonly used in redox titrations. A mixture of potassium ferrocyanide, potassium ferrocyanide, and phosphate buffered solution is used to provide a buffer for beta-galactosidase, which is used to cleave X-Gal, resulting in a bright blue visualization where an antibody (or other molecule) conjugated to Beta-gal has bonded to its target. When it reacts with Fe(3), it turns Prussian blue. As a result, it is used in laboratories as an identifying reagent for iron. Potassium ferrocyanide can be used as a fertilizer for plants. Prior to the invention of the castner process in 1900, potassium ferrocyanide was the primary source of alkali metal cyanides.[3] Potassium cyanide was produced in this historical process by decomposing potassium ferrocyanide.Potassium ferrocyanide is nontoxic and does not decompose in the body to cyanide. Rat toxicity is low, with a lethal dose (LD50) of 6400 mg/kg. Thus potassium ferrocyanide is the chemical compound with various uses and it has various sources of uses it may be hazardous if the fungus become resistant against potassium ferrocyanide. Potassium ferrocyanide uses can be reduced if this happens, so this study was carried out to check the effectiveness of potassium ferrocyanide against the various fungus found in soil since potassium ferrocyanide is also used in fertilizers, Potassium Ferrocyanide K4(Fe(CN)6) is used in the food and pharmaceutical industries for coloring purposes. The potassium ferrocyanide is used as a colorant in sugar, in food products such as baked goods, pasta, fruit, jelly and pie fillings, dairy products, meat and fish products, coffee, and chewing gum; and as a component in medicines and pharmaceuticals such vaccines, as vitamins, and antibiotics. It is commonly used in the manufacturing of dry-matter- free foods and food colorants, but is the increased use of foods contains small amounts of added potassiumferrocyanide. This colorant is not a additive and does not affect food safety. Potassium Ferrocyanide is usually added to sugar to improve the color of the food.Fungus occurs nearly everywhere in nature. [4] They present are mostly found in soil, fungus occur most abundantly Where they found moisture and a temperature suitable for their growth and multiplication. If the fungus found some of the way to be induced in potassium ferrocyanide the effectiveness of potassium ferrocyanide will be decreased.Thus the proper study and analysis are required in specific intervals of times to make the required changes for the effectiveness of the potassium ferrocyanide.[5]

1.1 Properties of Potassium Ferrocyanide

- Chemical formula :$K_4[Fe(CN)6]$
- Molar mass : 368.35 g/mol
- Appearance. : Light yellow, Crystalline granules
- Density : 1.85 g/cm³
- Boiling point : (decomposes)
- Solubility : Insoluble in ethanol, ether
- Magnetic susceptibility (X) : $-130.0.10^{-6}$ cm³/mol
- Hazard statements : H411

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Impact Factor: 5.781
Volume 3, Issue 12, December 2022

- Flash point : Non-flammable
- LD50 (median dose) : 6400 mg/kg (oral, rat)
- Other anions : Potassium ferricyanide
- Other cations : Sodium ferrocyanide Prussian blue

II. MATERIALS AND METHOD

Study Area: Study was carried out in ZSCT's Thakur shyamnarayan degree college, Kandivali East, Mumbai.

Sample Collection and Processing: Soil sample were collected from the two different farms near the virar station the soil sample were directly picked up and packed into the small plastic packets and the further study was investigated in microbiology laboratory of ZSCT's Thakur Shyamnarayan Degree College

Materials: The materials used include glass wares such as sterilePotato Dextrose Agarbottles, sterile Nutrient agar bottle, sterilebeaker, sterileconical flasks, measuring cylinder, glass slides, aluminium foil, cotton wool, sterileswab and spirit lamp.

Media/Agar:

- SterilePotato Dextrose agar
- SterileNutrient agar
- Composition of potato dextrose agar: (peptone 17g, proteose peptone 3g, lactose 10g, bile sales 1.5g,Sodium chloride 5g, agar 13.5g, neutral red 0.03g, crystal violet 0.001g, distilled water 1L, final Ph 7.1)
- Composition of Nutrient agar: (Distilled water 500ml, beef extract 0.5g, year extract 1g, peptone 5g, sodium chloride 2.5g, agar 7.5g)

Washing and Sterilizing of Materials: All the glassware instruments were first washed with the detergent and then they were air dried and then all the material and apparatus were autoclaved This was done to make all the material sterilized so there should be no microbial contamination. Preparation of medium for cultivation of fungus: The media used (sterileNA and sterilePotato Dextrose Agar) were weighed and prepared according to manufacturer's specification. The prepared media was carefully packed into the autoclave and sterilized at 121°C for 15 minutes. Prior to use, the media were cooled to about 45°C

Fungus Inoculation: The inoculation of Fungus from soil sample which was involved was the direct pouring of soil suspension on the nutrient agar and on potato dextrose agar in petri dishes each labeled according to date and source of sample code. The streaked sample was then incubated for 48 hours at 37°C after which the colonies were observed. After observing the growth the different colonies of fungus different types of Fungus were separated accordingly by streaking them on the different plates and incubating them for 48 hours at 37°C their growth were observed carefully.

ISSN (Online) 2582-9327

IJARSCMT

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Impact Factor: 5.781

Volume 3, Issue 12, December 2022

III. OBSERVATION

After the incubation of the plates for 48 hours at room temperature the following results were observed. They are as followed, all the plate in which the soil suspension was inoculated were observed carefully.The growth of the various fungus which were present in the soil suspension were observedand after the observation of growth we observed their colony and From the standard reference we identified the fungus which were present in the soil.

- *Alternariaalternata*
- *Aspergillusfumigatus*
- *Aspergillusflavus*
- *Candida albicans*
- *Lichtheimiahyalospora*
- *Candida tropicalis*
- *Rhizopusoryzae*
- *Aspergillusterreus*

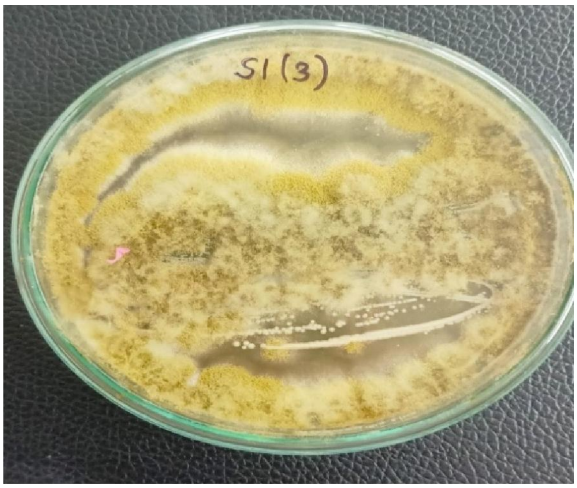
All the above mentioned fungal species were observed on the plates which was containing soil sample .

Isolation of fungus from soil sample 1

From Nutrient Agar plate

1) *Alternariaalternata. 2) Aspergillusfumigatus*

Alternariaalternata.



Aspergillusfumigatus

ISSN (Online) 2582-9327

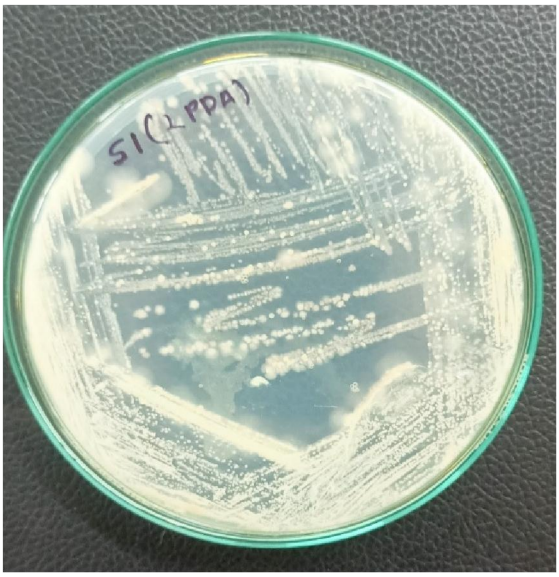
IJARSCMT

International Journal of Advanced Research in Science, Commerce, Management and Technology

Impact Factor: 5.781

Volume 3, Issue 12, December 2022

From Potato Dextrose Agar plate



1) Aspergillusflavus 2) Candida albicans

Aspergillusflavus. *Candida albicans*

Isolation of fungus from soil sample 2

From Nutrient Agar plate

1) Lichtheimiahyalospora. 2) Candida tropicalis

Lichtheimiahyalospora. *Candida tropicalis*

ISSN (Online) 2582-9327

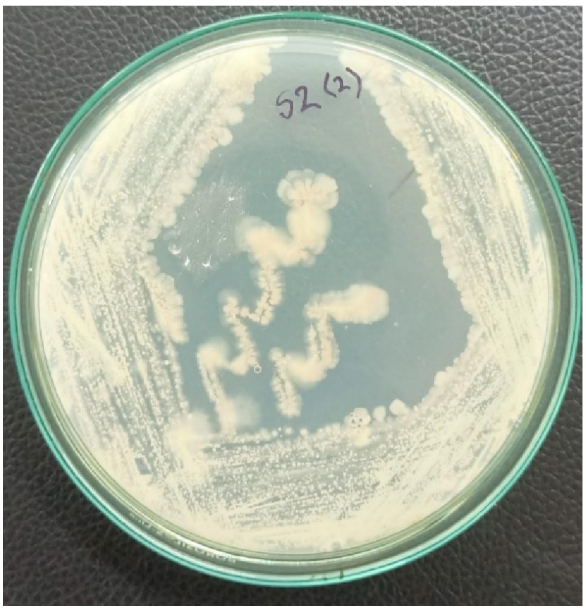
IJARSCMT

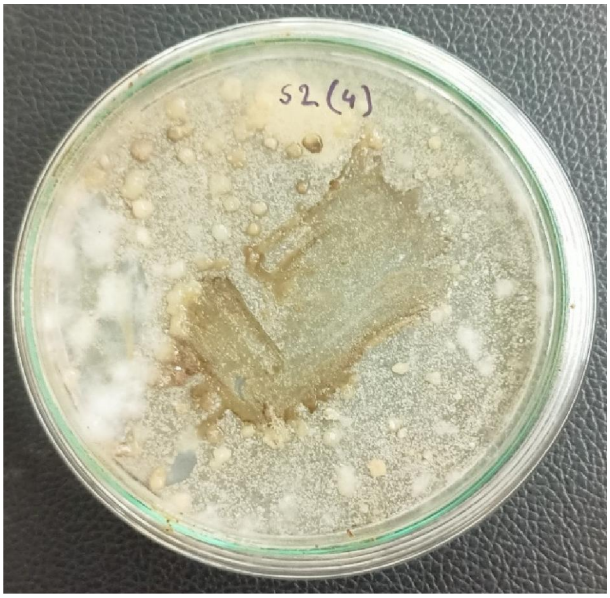
International Journal of Advanced Research in Science, Commerce, Management and Technology

Impact Factor: 5.781

Volume 3, Issue 12, December 2022

From Potato Dextrose Agar plate





1) Rhizopusoryzae. 2) Aspergillusterreus

Rhizopusoryzae. *Aspergillusterreus*

IV. RESULTS

The table given below is the result which has been observed in our experiment we observed the different types of fungus which were present in the soil which is also the common microfloraof the soil which were observed on the Nutrient agar plate and PotatoDextrose Agar plate

Result Table 1 of Soil sample 1

Medium	Results
1. SterileNutrient Agar	*Alternariaalternata*
2. Sterile Nutrient Agar	*Aspergillusfumigatus*
3. Sterile Potato Dextrose Agar	*Aspergillusflavus*
4. Sterile Potato Dextrose Agar	*Candida albicans*

Result Table 2 of Soil sample 2

Medium	Results
1. Sterile Nutrient Agar	Lichtheimiahyalospora
2. Sterile Nutrient Agar	Candida tropicalis
3. Sterile Potato	Rhizopusoryzae

ISSN (Online) 2582-9327
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Impact Factor: 5.781
Volume 3, Issue 12, December 2022

Dextrose Agar	
4. Sterile Potato Dextrose Agar	Aspergillusterreus

After the isolation of the fungus from the soil we have performed our main experiment which was to checking their resistivity and sensitivity against the various chemicals which are present in the fungicide, bactericide and other chemicals which have the ability to resist the growth of fungus.

V. METHODS OF CHECKING RESISTANCE AND SENSITIVITY OF FUNGUS

Firstly we have to prepare the chemicals plate (mainly containing the chemicals and agar which will be acting as our media for checking the resistance and sensitivity of the fungus.

VI. MATERIALS AND METHOD

The materials which were used in this experiment are as follows.

Apparatus:

Sr no	Apparatus	Quantity	Volume
1	Sterile empty petriplate	80	20ml
2	Sterile test tubes	10	18ml
3	Sterile conical flask	10	500ml
4	Nichrome wire loop	1	-
5	Test tube stand	2	-

Chemicals:

Sr no	Chemicals	Quantity
1	Sterile distilled water	3000 ml
2	Sterile saline	100 ml
7	Potassium ferrocynide	480gram

Agar medium:

Sr no	Agar medium	Quantity
1	Nutrient Agar	42 gram
2	Potato Dextrose Agar	84 gram
3	Agar Agar Powder	33 gram

Preparation Method: Firstly the Two Agar media were selected1) Sterile nutrient agar medium 2) Sterile potato dextrose agar medium, and then chemicals were mixed with distilled water according to their solubility in water and then the nutrient agar media and potato dextrose agar media were

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Impact Factor: 5.781
Volume 3, Issue 12, December 2022

prepared separately in the distilled water containing the chemicals and agar medium were prepared according to the manufacturer specifications.
Solubility of chemicals in water at room temperature.

- Potassium ferrocynide

Soluble in water at room temperature 80grams/100ml.

Preparation of different media containing chemical
Potassium ferrocynide media
Potassium ferrocynide nutrient agar media

8.4gram of nutrient agar powder was suspended in 300ml of distilled water. 240gram potassium ferrocynide was mixed and dissolved in distilled water and Sterilized by autoclaving at 121°C for 15 minutes. Liquid media was into the petri dish.
Potassium ferrocynide potato dextrose agar media

11.7gram of Potato dextrose agar powder and 3.3 gram of agar agarpowder was suspended in 300ml of distilled water. 240gram ofPotassium ferrocynide was mixed and dissolved in distilled water and sterilized by autoclaving at 121°C for 15 minutes. Liquid media was into the petri dish.

VII. INOCULATION OF FUNGUS ON CHEMICALS PLATE

- From the pure cultures of the fungus the inoculation were done on the two different medium (nutrient agar medium and potato dextrose agar medium) containing different types of chemical in petriplates.
- After the inoculation the plates were incubated at 37°C for 48 hours.

VIII. OBSERVATION

After the incubation period the following results were observed.
Observation for potassium ferrocynide

Name of Fungus sample	Nutrient Agar	Potato Dextrose Agar
1)Alternariaalternata	--	--
2)Aspergillusfumigatus	++	++
3) Aspergillusflavus	++	--
4) Candida albicans	--	--
5) Lichtheimiahyalospora	++	--
6) Candida tropicalis	--	--
7) Rhizopusoryzae	++	--
8) Aspergillusterreus	--	--

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Impact Factor: 5.781

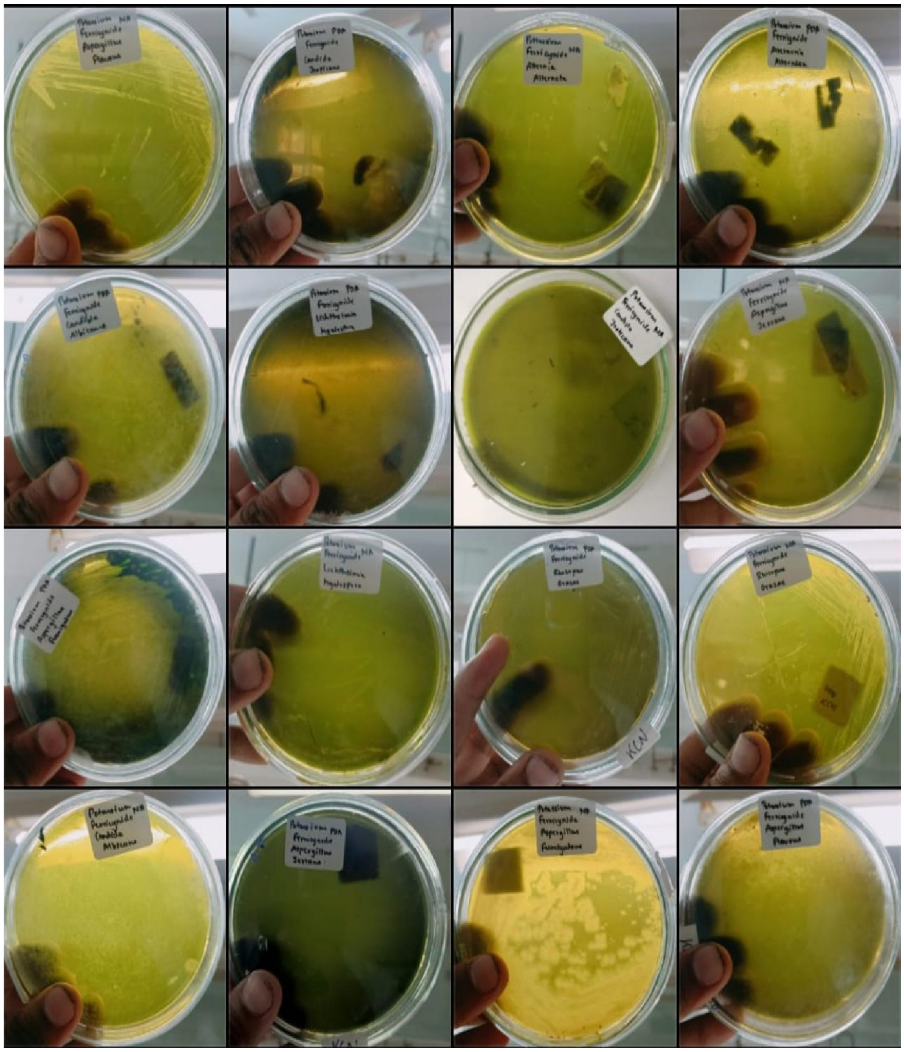
Volume 3, Issue 12, December 2022

IX. RESULTS

Results of Potassium ferrocynide

Aspergillusfumigatus, Aspergillusflavus, Lichtheimiahyalospora,Rhizopusoryzae were the fungus which were found to be resistant against the potassium ferrocynide on the nutrient agar media and Aspergillusfumigatus were found to be resistant on potato dextrose agar media containing Potassiumferrocynide.

The Photoplates for the above results are provided below



X. DISCUSSION

- Fertilizers which are important for the protection of the crops and the soilHowever they play an important role in the farming throughout the world wherever it's possible.[6]Fertilizers are the products that prevent or mitigate damage caused by microorganisms, typically fungi and bacteria, to other living organisms such as people, animals, plants including agricultural crops, as well as physical structures such as buildings and plant products.[7]
- It may be harmful if the fungal species became resistant to the fertilizers etc.

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Impact Factor: 5.781

Volume 3, Issue 12, December 2022

- The multi-resistant strain of fungus which are resistant against variousfertilizers which occurs in the soil may be harmful for the crops and they have potential to destroy and damage thecrops.
- In histology, potassium ferricyanide is used to detect ferrous iron in biological tissue. Potassium ferricyanide reacts with ferrous iron in acidic solution to produce the insoluble blue pigment, commonly referred to as Turnbull's blue or Prussian blue,[8] if the fungus become resistant then there is the possibility of the false results which may be harmfulif fungus found way into it.[9]
- Potassium ferrocyanide treatment poses safety and environmental protection problems, but is absolutely necessary for the efficient removal of iron from wines rich in this cations, there are various organism which are founded in the wines if they have negative impact on the potassium ferrocyanide this may be hazardous for this.

XI. CONCLUSION

This experiment was carried out using potassium ferrocyanide which is present in the fertilizers,wines, in mining industry etc. If the fungus becomes resistant to the potassium ferrocyanide it is of no use, the importance of the potassium ferrocyanide will be retarded.

Key Conclusion

- Potassium ferrocyanide was resistant against the potassium ferrocyanide were *Aspergillusfumigatus, Aspergillusflavus, Lichtheimiahyalospora, Rhizopusoryzae*. etc
- These fungus are present in the soil naturally however if they can be transmitted from one place to another with the help of various vector which are present in the environment
- Potassium ferrocyanide is of great importance they have wide variety of uses if these species of the fungus become resistant against the potassium ferrocyanide there may be negotiable uses of potassium ferrocyanide.
- They are also use in citric acid production if citric acid get contaminated with these fungal species in which the potassium ferrocyanide is used these fungal species have the ability to degrade the citric acid fermentation process.

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