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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Seasonal Distribution of different Mycoflora from Proposed KOSI – MECHI River Interlinking Canal

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**ABSTRACT**

*Fungal vegetation is greatly affected in enrouted region of proposed KOSI – MECHI river interlinking project in kharif season due to problem of water scarcity. A total of 14 different species were isolated from 20 different areas of Araria, Kishanganj, Purnea and Katihar district of Bihar. Total 1140 isolates of different species were obtained during May to April. Aspergillus, Rhizopus and Alternaria show the highest percentage of contribution. It was observed that, during October to January (In Rabi season) the fungal isolates were highest in number and during May to August (In Kharif Season) the fungal isolates were minimum in number. This observation conducted that; these areas were facing major issue of water scarcity in Kharif season.*
*Keywords: Kosi,Mechi, Eastern Kosi Main Canal (EKMC),Mycoflora*

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## INTRODUCTION

The river Kosi is an international river originating from Tibet flowing through Tibet, Nepal in Himalayan Mountains, and the lower portion through plains of North Bihar. To overcome the acute problem of shifting of course of Kosi River, heavy sediment load, flooding etc; the interlinking of KOSI-MECHI river has been proposed. It also helps to improve the agricultural production.

The Kosi project includes a barrage namely Hanuman Nagar across river Kosi located near Hanuman Nagar town close to Indo-Nepal border, canal headworks, Western Kosi Main Canal (WKMC) system in Nepal, Eastern Kosi Main Canal (EKMC) system in India. The present proposal is an extension of EKMC up to river Mechi, a tributary of river Mahananda. The canal – meant to connect two rivers in Bihar, will cross thirteen rivers on its path: Parman, Tehri, Lohandra, Bhalua, Bakra, Ghaghi, Pahara, Nona, Ratua, Kawal and Kankai. The aim of extension of EKMC up to Mechi river is mainly to provide irrigation benefits in new unirrigated area to the water scarce Mahananda basin command in the districts of Araria, Kishanganj, Purnea and Katihar during kharif season depending upon the pondage available in Hanuman Nagar barrage. The new culturable area covered by the link project for irrigation is 2,14,812 ha utilizing 1718 MCM of water.

Fungi are very successful inhabitants of soil, due to their high plasticity and their capacity to adopt various forms in response to adverse or unfavourable conditions [1]. The diversity and activity of fungi is regulated by various biotic (plant and other organism) and abiotic (soil, pH, moisture, salinity, structure and temperature) factors [2,3]. Fungi can be found in almost every environment and can be live in wide range of pH and temperature [4]. Fungi participate in nitrogen fixation, hormone production, biological control against root pathogen and protection against drought [5,6]. They also play an important role in stabilization of soil organic matter and decomposition of residues [7]. Therefore, the aim of this study is to find out the impact of irrigated and new unirrigated area on micro floral diversity.

## MATERIAL AND METHODS

### Study Site and Locations

The study was conducted in Enrouted area via Araria, Kishanganj, Purnea and Katihar district of Bihar of KOSI-MECHI interlinking project. Five different sites of all four district were selected.

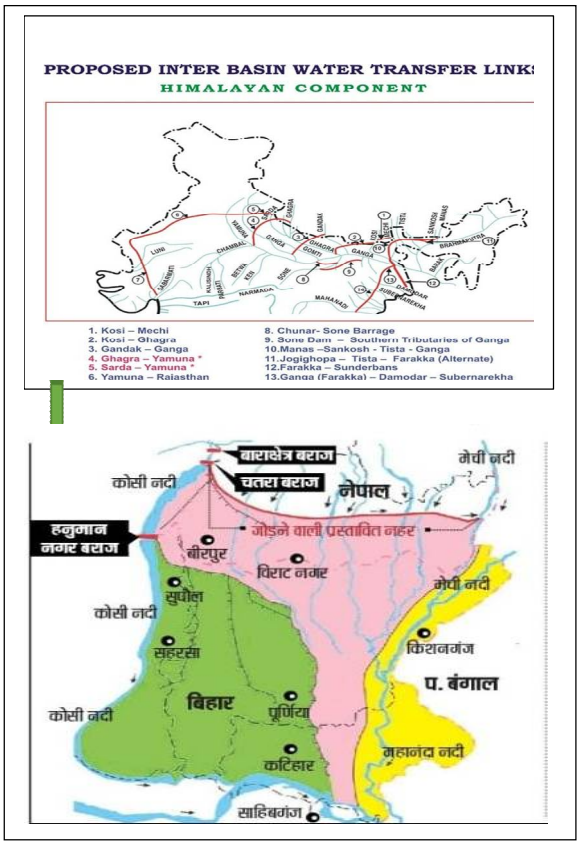


**Figure 1:**Study site and location.

**Collection of Soil Samples**

Ten soil samples from different crop fields of each site of proposed KOSI-MECHI enroute canal were collected (Table-1). Soil was collected from a depth of 15cm and are kept in sterilized polyethylene bags. Each sample was labelled properly by indicating the site of collection, date, and place of collection. The sampling was done during May 2021 to April 2022, and focused on Rabi season and Kharif season.

**Determination of Physio-chemical Parameters of the Soil Samples**

The physio-chemical parameters of the collected soil samples (Table2) such as, moisture content, organic carbon, nitrogen, pH, exchangeable potassium, calcium, potassium, calcium, magnesium and sodium were analyzed [8,9].

**Isolation of Fungi**

Serial dilution method was used to isolate the fungal colonies from soil samples [10]. For this, 25 grams of soil were added to 250 ml of sterile water in a flask and shake vigorously. Then transfer 10 ml of dilution with a sterile pipette to 90 ml of sterile water and repeat the process to obtain dilution up to 1:1000. Transfer 1 ml from last three dilutions to each of 3 sterile petri dishes containing PDA (Potato Dextrose Agar) media with chloramphenicol to inhibit the bacterial growth. Incubate at 26±2°C for 5 to 7 days. Transfer each colony of different isolated fungi on PDA media to obtain pure colony of different species of fungi.

**Identification of Fungi**

Fungal Colonies were identified on the basis of morphological and microscopic characters [11]. Colour and growth pattern of each species was used for morphological identification (Fig 2). For microscopic identification, slides where prepared from the colonies and examined under microscope (Table 3).The laboratory manual of Barnett and Hunter [12] was used for microscopic identification.

**Statistical Analysis**

Number of colonies per plate in 1 gm of soil was calculated to have the percent contribution of each isolate and the frequencies of occurrences of each individual isolate as well as the genus were calculated using the formula as expressed below:

$$\text{Frequency of occurrence} = \frac{\textbf{Number of individual species isolated}}{\textbf{Total number} \text{ of all species isolated}} \times 100$$

Where

CFU = Colony Forming Unit

$$\% \text{ Contribution} = \frac{\text{Total No. of CFU an individual species}}{\text{Total no. of CFU of all species}} \times 100$$

## RESULT AND DISCUSSION

During the study period, 14 fungal species were isolated from different area of proposed interlinking Canal region of KOSI-MECHI river (Table4). The most frequent genera isolated were *Aspergillus* and *Rhizopus* followed by *Alternaria* and *Fusarium*. *Penicillium*, *Trichoderma* and *Neurospora* were observed in lesser frequency (Table5). Frequency wise distribution of flora in different month (Fig.3) revealed that in Kharif season (during May to July) minimum colony of fungal species were observed followed by Rabi season (Oct to Dec).

**Table 1:** Soil samples collected from different locations of proposed KOSI – MECHI Enroute Canal.

| District | Sample Code | Nature of Field | Sampling Site | Location | | Soil Type |
|---|---|---|---|---|---|---|
| | | | | Latitudes | Longitudes | |
| Araria | Ar-1 | Wheat | Mirganj | 26.368151° | 87.240640° | Sandy |
| | Ar-2 | Rice | Bhorha Ghat | 26.306565° | 87.420311° | Sandy loam |
| | Ar-3 | Maize | Doria | 26.289918° | 87.433425° | Sandy clay loam |
| | Ar-4 | Rice | Dhowabari | 26.363941° | 87.468306° | Sandy loam |
| | Ar-5 | Black Gram | Bahadurganj | 26.261639° | 87.815485° | Sandy clay loam |
| Katihar | Ka-1 | Rice | Sarifnagar | 25.517521° | 87.565724° | Sandy loam |
| | Ka-2 | Maize | Barari | 25.340783° | 87.096916° | Sandy |
| | Ka-3 | Rice | Mansahi | 25.474194° | 87.588519° | Sandy loam |
| | Ka-4 | Wheat | Taiyabpur | 25.714193° | 87.807762° | Sandy |
| | Ka-5 | Potato | Bhaunagar | 25.662043° | 87.817172° | Sandy loam |
| Kishanganj | Ki-1 | Rice | Khoragachhi Miki | 26.332153° | 87.570802° | Sandy |
| | Ki-2 | Wheat | Pohara | 26.401821° | 87.525072° | Sandy |
| | Ki-3 | Maize | Rampur | 26.372699° | 87.663324° | Sandy loam |
| | Ki-4 | Jute | Kanchoribari | 26.393127° | 87.757947° | Sandy |
| | Ki-5 | Jute | Parbhata | 26.352153° | 87.530802° | Sandy loam |
| Purnea | Pu-1 | Rice | Suwara | 25.796860° | 87.511451° | Sandy loam |
| | Pu-2 | Jute | Ramnagar | 25.769880° | 87.481655° | Sandy clay loam |
| | Pu-3 | Wheat | Baisa | 26.035056° | 87.802604° | Sandy loam |
| | Pu-4 | Jute | Kadwa | 25.695234° | 87.753213° | Sandy clay loam |
| | Pu-5 | Rice | Amour | 25.749954° | 87.468445° | Sandy loam |

This is due to the soil vegetation, water holding capacity, pH and nutrient medium of soil. In kharif season the nutrient depletion and dryness of soil reduce the fungal population (Fig.3). The species which have high tolerance capacity to different climatic condition were mainly shows their colonization in this season. Due to lack of water, there was less distribution of vegetation in enrouted area of proposed to KOSI-MECHI river interlinking Canal in kharif season. It affects the soil nutrient value as well as agricultural production because fungal diversity determines plant biodiversity and productivity [13].

**Table 2:**Physico-chemical properties of the soil collected from Canal area between May 2021 to April 2022. (Mean Value)

| Sites Code | pH | Moisture (%) | OC (%) | N (%) | Ca (Cmol/Kg) | Mg (Cmol/Kg) | K (Cmol/Kg) | Na (Cmol/Kg) |
|---|---|---|---|---|---|---|---|---|
| **Ar-1** | 7.4 | 1.0 | 0.32 | 0.06 | 1.25 | 0.60 | 0.46 | 0.43 |
| **Ar-2** | 7.5 | 1.0 | 0.56 | 0.07 | 1.35 | 0.60 | 0.56 | 0.48 |
| **Ar-3** | 8.3 | 1.5 | 1.08 | 0.10 | 1.05 | 1.33 | 0.87 | 0.74 |
| **Ar-4** | 7.2 | 2.0 | 1.24 | 0.11 | 1.52 | 1.53 | 1.45 | 0.34 |
| **Ar-5** | 6.9 | 2.1 | 0.22 | 0.04 | 1.22 | 0.53 | 0.45 | 0.43 |
| **Ka-1** | 8.4 | 1.5 | 1.18 | 0.11 | 1.15 | 1.23 | 0.81 | 0.70 |
| **Ka-2** | 7.3 | 1.1 | 0.22 | 0.06 | 1.23 | 0.70 | 0.49 | 0.78 |
| **Ka-3** | 7.8 | 1.9 | 1.08 | 0.12 | 1.04 | 1.13 | 0.77 | 0.64 |
| **Ka-4** | 6.8 | 1.7 | 1.28 | 0.10 | 1.05 | 1.06 | 0.67 | 0.84 |
| **Ka-5** | 7.4 | 1.6 | 1.10 | 0.13 | 1.24 | 1.44 | 0.37 | 0.77 |
| **Ki-1** | 8.6 | 1.5 | 1.18 | 0.10 | 1.05 | 1.27 | 0.37 | 0.94 |
| **Ki-2** | 6.9 | 1.1 | 0.52 | 0.04 | 1.52 | 1.53 | 1.45 | 0.34 |
| **Ki-3** | 7.1 | 2.1 | 0.22 | 0.14 | 1.22 | 0.58 | 0.49 | 0.43 |
| **Ki-4** | 7.3 | 2.1 | 0.44 | 0.03 | 1.33 | 1.65 | 0.88 | 0.74 |
| **Ki-5** | 6.9 | 2.1 | 0.22 | 0.04 | 1.22 | 0.53 | 0.45 | 0.43 |
| **Pu-1** | 7.3 | 1.0 | 0.31 | 0.05 | 1.24 | 0.62 | 0.47 | 0.46 |
| **Pu-2** | 7.5 | 2.0 | 0.65 | 0.21 | 1.48 | 1.23 | 1.43 | 0.76 |
| **Pu-3** | 6.8 | 1.4 | 0.41 | 0.10 | 1.25 | 0.62 | 0.46 | 0.43 |
| **Pu-4** | 6.4 | 1.1 | 0.52 | 0.14 | 1.52 | 1.53 | 0.85 | 0.98 |
| **Pu-5** | 8.3 | 1.4 | 1.08 | 0.10 | 1.05 | 1.27 | 0.81 | 0.70 |

**Table 3: Occurrence of Fungal species in Different area of canal region**

| Sl. No. | Colony Texture | Microscopic Character | Species Identification |
|---|---|---|---|
| 01 | Black and pale-yellow colour | Conidiophores are long, Smooth and hyaline; Biseriate and radial conidia; septate hyphae | *Aspergillus niger* |
| 02 | Yellow-green Colsony | Long conidiophores; Biseriate; globose, Smooth | *Aspergillus flavus* |
| 03 | Orange-Cream colony | Conidial head are columnar and uniseriate; short, smooth- walled conidiophore; globose conidia | *Aspergillus fumigatus* |
| 04 | Yellow-white Colony | Conidia are smooth and oval; philades uniseriate; rough conidiophore | *Aspergillus oryzae* |
| 05 | Light brown colony | Brown, erecti conidiophore; obclavate, smooth, branched, transverse and longitudinal septate conidia | *Alternaria alternata* |
| 06 | Dark brown colony | Brown conidiophore; transverse septate branched obclavate conidia | *Alternaria solani* |
| 07 | Pinkish white colony | Macroconidia septate, curved, irregular branched conidiophore | *Fusarium oxysporum* |
| 08 | White colony | Irregular branched conidiophore; sickle shaped, septate conidia | *Fusarium solani* |
| 09 | Green colony with 2-3 concentric ring | Branched conidiophore; flask shaped philades; green globose and smooth conidia | *Trichoderma harzianum* |
| 10 | White green colony with 2 concentric rings | Highly branched, symmetrical conidiophore; dark green and ovoidal conidia; branched philades | *Trichoderma asperellum* |
| 11 | White green colony with 1-2 concentric rings | Conidiophore are branched at 90° angle; single philades; globose and smooth conidia | *Trichoderma viride* |
| 12 | Grey - green colony | Green, oval, smooth conidia; ampulli form philades; brush shaped conidiophore | *Penicillium chrysogenum* |
| 13 | Grey fuzzy colony | Black round spore; branched mycelia | *Rizopusstolonife* |
| 14 | Orange pink cottony colony | Axon like mycelia; oval shaped conidia | *Neurospora crassa* |

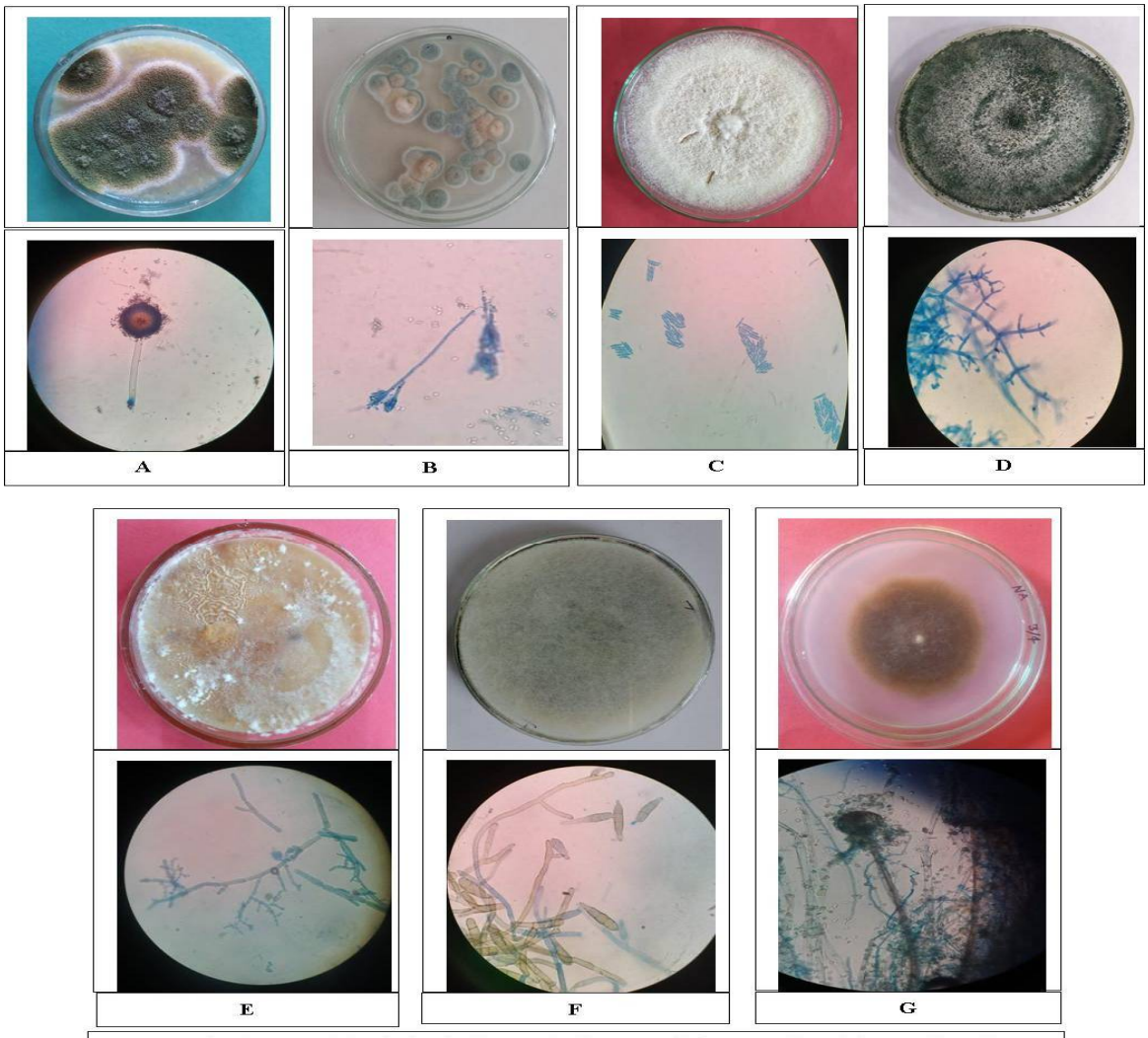


**Figure 2:**Showing morphological and microscopic diagram of (A) *Aspergillus*, (B) *Penicillin*, (C) *Fusarium*, (D) *Trichoderma*, (E) *Neurospora*, (F) *Alternaria*, (G) *Rhizopus*.

**Table 4:** Total number of the different fungal species isolated from all sampling sites.

| Number of isolates of individual species | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **District** | **Site** | ***Aspergillus*** | | | | ***Alternaria*** | | ***Fusarium*** | | ***Rhizopus*** | ***Trichoderma*** | | | ***Penicillium*** | ***Neurospora*** | **Total** | |
| | | *A. N.* | *A. fl* | *A. fu* | *A. ory* | *A. alt* | *A. sol.* | *F. o.* | *F. so* | *R. sto.* | *T. har.* | *T. asp* | *T. vir* | *P. chy.* | | | |
| **Araria** | Ar-1 | 20 | 25 | 11 | 10 | 13 | 8 | 8 | 1 | 13 | 1 | 0 | 0 | 6 | 0 | **116** | **410** |
| | Ar-2 | 1 | 17 | 8 | 8 | 1 | 3 | 1 | 0 | 7 | 0 | 1 | 4 | 0 | 0 | **51** | |
| | Ar-3 | 12 | 28 | 4 | 1 | 8 | 2 | 2 | 0 | 12 | 2 | 0 | 0 | 2 | 0 | **73** | |
| | Ar-4 | 17 | 16 | 3 | 19 | 2 | 1 | 7 | 0 | 10 | 0 | 2 | 0 | 0 | 1 | **78** | |
| | Ar-5 | 19 | 23 | 12 | 2 | 6 | 0 | 10 | 2 | 17 | 0 | 0 | 1 | 0 | 0 | **92** | |
| **Katihar** | Ka-1 | 7 | 12 | 0 | 4 | 0 | 11 | 6 | 0 | 1 | 0 | 0 | 0 | 3 | 0 | **44** | **250** |
| | Ka-2 | 10 | 1 | 8 | 0 | 6 | 2 | 12 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | **44** | |
| | Ka-3 | 10 | 21 | 2 | 0 | 0 | 8 | 1 | 0 | 12 | 1 | 3 | 0 | 1 | 0 | **69** | |
| | Ka-4 | 13 | 4 | 3 | 0 | 1 | 0 | 8 | 8 | 1 | 1 | 0 | 0 | 0 | 2 | **41** | |
| | Ka-5 | 3 | 12 | 0 | 3 | 4 | 2 | 2 | 22 | 3 | 0 | 1 | 0 | 0 | 0 | **52** | |
| **Kishanganj** | Ki-1 | 8 | 3 | 1 | 4 | 8 | 2 | 0 | 1 | 4 | 4 | 0 | 0 | 2 | 0 | **37** | **203** |
| | Ki-2 | 6 | 5 | 0 | 1 | 2 | 0 | 2 | 0 | 12 | 0 | 2 | 1 | 0 | 0 | **31** | |
| | Ki-3 | 12 | 7 | 0 | 1 | 3 | 0 | 0 | 2 | 7 | 0 | 0 | 2 | 0 | 0 | **34** | |
| | Ki-4 | 7 | 8 | 1 | 1 | 12 | 1 | 0 | 0 | 21 | 0 | 0 | 0 | 4 | 0 | **55** | |
| | Ki-5 | 8 | 10 | 0 | 2 | 6 | 1 | 6 | 0 | 11 | 0 | 1 | 0 | 0 | 1 | **46** | |
| **Purnea** | Pu-1 | 22 | 8 | 8 | 1 | 4 | 13 | 11 | 0 | 10 | 1 | 0 | 1 | 10 | 0 | **89** | **277** |
| | Pu-2 | 7 | 2 | 1 | 3 | 0 | 1 | 0 | 1 | 13 | 0 | 1 | 0 | 2 | 0 | **31** | |
| | Pu-3 | 3 | 12 | 6 | 1 | 1 | 0 | 5 | 0 | 21 | 0 | 0 | 1 | 0 | 0 | **50** | |
| | Pu-4 | 5 | 17 | 4 | 1 | 0 | 1 | 2 | 8 | 10 | 0 | 1 | 0 | 8 | 2 | **50** | |
| | Pu-5 | 1 | 14 | 3 | 2 | 3 | 9 | 1 | 0 | 14 | 0 | 0 | 1 | 0 | 0 | **48** | |
| | | **191** | **245** | **75** | **64** | **80** | **65** | **84** | **45** | **203** | **10** | **12** | **12** | **48** | **6** | **1140** | |

**Table 5:** Frequency of different fungal species isolated during May 2021 to April 2022.

| Sl. No. | Identified fungi | Occurrence times | Frequency occurrence (%) |
|---|---|---|---|
| | ***Aspergillus* species** | **575** | **50.44** |
| 1. | *A. niger* | 191 | 16.75 |
| 2. | *A. flavus* | 245 | 21.49 |
| 3. | *A. fumigatus* | 75 | 06.58 |
| 4. | *A. oryzae* | 64 | 05.62 |
| | ***Alternaria* species** | **145** | **12.72** |
| 5. | *Alternaria alternata* | 80 | 07.02 |
| 6. | *A. longipes* | 65 | 05.70 |
| | ***Fusarium* species** | **129** | **11.32** |
| 7. | *F. oxysporum* | 84 | 07.37 |
| 8. | *F. solani* | 45 | 03.95 |
| | ***Rhizopus* species** | **203** | **17.80** |
| 9. | *R. oryzae* | 203 | 17.80 |
| | ***Trichoderma* species** | **34** | **02.98** |
| 10. | *T. harzianum* | 10 | 0.88 |
| 11. | *T. asperellum* | 12 | 01.05 |
| 12. | *T. viride* | 12 | 01.05 |
| | ***Penicillium* species** | **48** | **04.21** |
| 13. | *P. oxalicum* | 48 | 04.21 |
| | ***Neurospora* species** | **06** | **0.53** |
| 14. | *N. crassa* | 06 | 0.53 |
| **Total Isolates** | | **1140** | **100** |

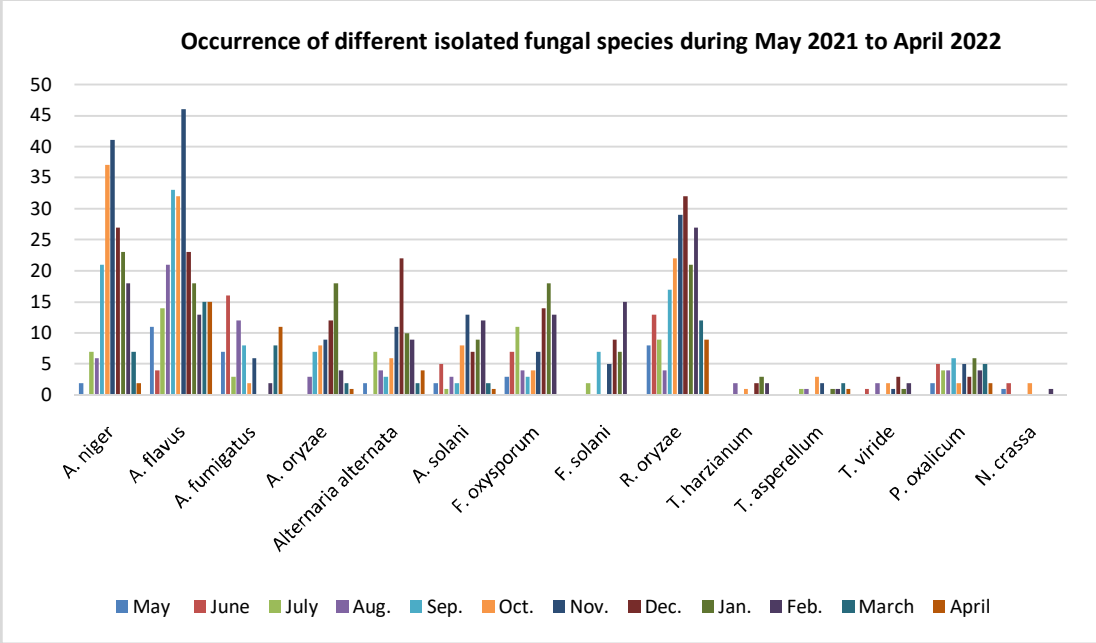


**Figure 3:** Showing number of different fungal species isolated during May 2021 to April 2022

## CONCLUSION

This study revealed that, the agricultural land area of proposed interlinking canal of KOSI- MECHI river is facing major issue of humidity and soil nutrient quality in Kharif season. It decreases the amount of kharif crop production. To overcome this problem, adequate amount of water is needed in kharif season. Therefore, KOSI-MECHI interlinking project is a worthwhile initiative in the agricultural sector.

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## CONFLICTS OF INTEREST

There is no conflict of interest

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