Isolation of the soil mycoflora, their characterization and identification were done by following standard methods. Antimicrobial activities of crude methanolic extracts of *Penicillium* isolates were done by agar disc diffusion method against clinical bacterial pathogens as per CLSI norms. The metabolite of the most bioactive *Penicillium* isolate (*P. chrysogenum*, P06) was purified through chromatographic method purified compound was further preceded for determination of Minimum inhibitory concentration (MIC) and HPLC analysis. Three potential species producing coloured secondary metabolites were further studied to optimize the biomass and pigment production in different laboratory based media by standard methods.

Native Penicillia from undisturbed natural soil from North-eastern region were identified and enumerated and their relative densities, frequency and abundance were determined. From seven different sampling sites, a total of 252 different isolates of *Penicillium* were isolated which were accommodated 32 different species. Apart from all, twenty two genuses of different soil fungi including *Penicillium* were isolated from all the sampling sites. *Penicillium* and *Aspergillus* were dominant in all soil samples although their relative densities were varied considerably in different seasons. Species of *Penicillium* and *Aspergillus* were the most common (frequency 100%) and dominant species but their relative densities varied considerably. The relative density of *Penicillium* was higher in all the sites except in summer seasons where the relative density of *Aspergillus* was higher.
The total fungal population found in the studied sites were 98.87 (±10.65594) x10^3 CFU/g dry soils in all the seasons. The total CFU of *Penicillium* were also highest among the species in almost all the sites, average 18.73 (±1.101784) x10^3 CFU/g dry soil (n=7) where 27.2 x10^3 CFU/g in summer and 11.6 x10^3 CFU/g dry soil in winter followed by *Aspergillus* sp. Highest number of CFU of *Penicillium* spp. was recorded in S2 in summer seasons in top soil followed by autumn, pre monsoon and winter respectively.


Shannon-Weaver biodiversity index (H) of *Penicillium* was highest in the rainy seasons. There was also a significant correlation of H index and evenness among the sites in different seasons. The biodiversity index *i.e.* H index of some common species were *P. chrysogenum* (0.17), *P. oxalicum*, *P. janthinellum* (0.16), *P. citrinum*, *P. purpurogenum* (0.15), *P. digitatum*, *P. italicum* (0.14) etc. The total H index was highest in S3 (20.15). It has been observed that the soil nutrients characteristics in all
the sites were significantly different. The soil pH affected the population structure of the *Penicillium* species together with the associated micro flora. The average soil pH of the sample collected sites was 6.27 (±0.34) with the maximum of 6.6. The soil moisture content was 73.68 (±5.50) %, soil organic carbon 0.79 (±0.122) %, available nitrogen 0.28 (±0.07) %, Phosphorus 0.006 (±0.0007) % and Potassium 0.005 (±0.001) %. There was a positive correlation with the soil moisture and organic carbon content, soil pH and available phosphorus and potassium, organic carbon and available nitrogen among the sample collected sites. Soil pH and moisture was the most dependent factor for diversity indices and the optimum pH was recorded at 6.5 \( (R^2 \geq 0.67; P \leq 0.01) \). However, the diversity indices were not linear in different soil pH and other parameters. Higher soil organic carbon also favoured the population growth of *Penicillium* species.

Among the 32 species of *Penicillium*, a few species had shown antimicrobial activity against the tested bacterial pathogens. The isolates P03, P05, P06, P10, P14, P19, P23, P25 and P38 had shown antimicrobial activity against different tested bacterial pathogens. All the bacterial species were sensitive to P23 and P06 although their ranges of activity showed considerable variations. *S. bombycis* and *E. coli* was more sensitive to P06 and P23 cultural filtrate with an inhibition zone of more than 10mm in diameters followed by P25 cultural filtrates (≥7mm). *B. subtilis* was sensitive to P06 with the zone of inhibition of more than 10mm. *S. bombycis* was sensitive to P23, P25 (≥10 mm), P06 (≥7mm), P14 and P03 (≥4mm) isolates. *A. salmonicida* was sensitive to the P06, P23 and P10 isolates.

The methanolic extracts of the most active *Penicillium* isolates *i.e.* P. *chrysogenum* (P06) was purified by chromatographic method (TLC, Rf value 0.82) and
identified as Penicillin G by HPLC analysis. The purified compound was bio-assayed and the MIC of the pure compound was recorded against *B. subtilis* (25±2 µg /ml), *S. aureus*, *E. coli* and *K. pneumoniae* (30±2 µg /ml), *P. aeruginosa* (40±2 µg /ml) and *A. salmonicida* (45±2 µg /ml).

Jaccard’s genetic dissimilarity co-efficient varied from 0.00 to 0.87 among the *P. chrysogenum* isolates. The highest genetic dissimilarity co-efficient (0.833-0.875) was observed between *P. chrysogenum* Pc25, Pc09, Pc 10, Pc15 and Pc09 while the lowest value (0.00) was measured between six pair wise species combinations i.e. they were similar to each other. Iso-enzymes banding pattern of *P. chrysogenum* isolates showed that a total of 4 isozyme bands were formed after activity staining for enzyme α-esterase from all the samples. There were no variation in band number and position in activity staining of peroxidase. However, genetic variance among the isolates was found for α-esterase enzyme activity staining.

The growth rates of the isolates of *Penicillium* on seven agar media varied significantly. P001 grew better on CDA and MEA while for P002, YEA was the best for colony growth. The average colony diameter of P001 was 3.8±0.61cm with the maximum on CDA i.e. 4.5±0.20cm. The highest number of spores per cm² was obtained on CDA (94±5.6x10⁵ /cm²). CD broth medium was the best for mycelial fresh and dry weight for P001, P002 and P003 (1.00±0.05, 0.90±0.01 and 0.79±0.02 g/100ml respectively) followed by PDB (0.75 g, n=3) and OM (0.72g, n=3) medium. There was significant correlation between the mycelial growth and pigment production in different medium (r²=0.9, P<0.001). The highest fungal biomass (0.82±0.02g/100ml) and pigment production (P001, 890±50.6) was observed when initial pH of culture medium
was set at pH 6.0 in PD medium at 25°C. Nonlinear regression curve was fitted with the dependent parameters i.e. pH and temperature with pigment production and the results was highly significant ($P \leq 0.001$). By optimization of culture conditions of *Penicillium* isolates, red pigment production can be improved by seven fold under submerged fermentation. The higher concentration of pigment production by *Penicillium* sp. favours for commercial production of pigments.

It appears that undisturbed soils in the study area have considerable diversities of mould flora. Such microbial resources can be exploited for production of new active molecules like antibiotic and other useful pharmaceutical products.