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Isolation and characterization of pigment producing fungi

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ABSTRACT
The current study aimed to isolate and identify different fungal isolates and investigate their potential to produce pigments. From the current study, thirty fungal species belonging to ten genera were isolated from rhizosphere, rhizoplane, phyllosphere and phylloplane of four plants (*Medicago sativa* L., *Triticum aestivum* L., *Zea mays* L., and *Vicia faba* L.). *Aspergillus* was the most common fungus followed by *Penicillium*. The results showed that 19 (out of the 30 tested fungal isolates) exhibited various degrees of pigment production on Sabouraud Dextrose Broth medium. The yellow colored fungal pigments were produced by *Aspergillus flavus*, *Aspergillus niger*, *Emericella nidulans*, *Eurotium chevalieri*, *Penicillium chrysogenum* and *Penicillium citrinum.* Whereas, brown colored fungal pigments were produced by *Aspergillus terreus*, *Aspergillus ochraceus* and *Alternaria alternata*, as well, orange pigment was produced by *Epicoccum nigrum* and red pigment was produced by *Penicillium purpurogenum*. Only seven pigment-producing fungal isolates were considered as high producers (conc. > 3g/L), of which, *Penicillium purpurogenum* (PP2) was the highest producer of fungal pigments, yielding 5.156 g/L. So *Penicillium purpurogenum* is considered as promising fungal strain for production of natural pigments that may provide a viable green alternative to the current sources of pigments for the use in prospective food industry and industry of textiles.

INTRODUCTION

Pigments can be produced naturally or synthesized chemically and they have a chromophore coloring group. These pigments either directly produce color by light absorption in the visible area wavelength range or transfer energy to color intensifiers called auxochromes [1, 2]. Synthetic colorants pose a risk on human health by contributing to the emergence of mental disease, allergies, and a variety of malignancies [3] . As a result, there is an increasing need for eco-friendly, non-toxic colorants, particularly for applications where human health is at risk, such as coloring of food and the dying of children's clothes and leather clothing [4]. Natural pigments, that are derived from a variety of biological sources, including plants, animals, insects, and

microorganisms, offer a viable alternative source for coloring [5-8]. Microorganisms have many advantages for pigment production over plants and animals since their cells are much smaller and grow at a faster rate in inexpensive culture medium [9]. In addition, microorganisms have high productivity, and can yield a product throughout the year [10]. Moreover, Various microorganisms, such as algae, bacteria, fungi, and protozoa, produce natural pigments, such as carotenoids, flavins, melanin, quinines, monascin, phycocyanin, or indigo [11]. fungi can produce the widest range of soluble colors on a variety of substrates and environmental circumstances. These pigments are produced by fungi as secondary metabolites due to nutritional scarcity [12] or to help in improving fungal survival [13]. Many fungal species of the genus *Aspergillus* and *Penicillium* have a high potentiality to produce natural pigments [13-17]. The selection of a suitable strain, the fermentation process, as well as the choice of appropriate substrates or media, all play a role in increasing the productivity of fungal pigments [11]. The majority of fungal pigments are used economically as food, feed, and medicine colorants or as important nutrients. The pigment production by microorganisms may enhance the pharmaceutical, food, and feed industial biotechnology. The rapid development lead to the demand of colors in biotechnology related to food, textile, medicine and cosmetics. Microorganisms have potential to produce variety of metabolites which show biological and pharmacological activities. Microbial pigments show great antioxidant, anticancer and antimicrobial activities. Amongst microbes, fungi have great potential to release such metabolites which carry biological and pharmacological activities [16]. "The aim of the current study was to isolate rhizosphere, rhizoplane, phyllosphere and phylloplane fungi from four cultivated plants, *Medicago sativa, Triticum aestivum, Zea mays* and *Vicia faba* collected from Assiut Governorate. Furthermore, the current study was extended to investigate the biodiversity and fungal groups associated with collected plants as well to assess the potentiality of the isolated fungi to produce pigments.

MATERIALS AND METHODS

Selected area and sample collection

Four samples were collected from each of the following plant species; *Medicago sativa* L., *Triticum aestivum* L., *Zea mays* L., and *Vicia faba* L. were collected from the farm of Faculty of Sciences, Assiut University, Assiut Governorate, Upper Egypt. The collected plants and soil were placed in clean, sterile plastic bags and directly transferred into laboratory for isolation and identification of fungi.

Isolation of fungi from rhizosphere

The collected plants were detached and firmly shaken to remove extra soils and rhizosphere soils were used for isolation of rhizosphere fungi. on potato dextrose agar medium (PDA) supplemented with rose-bengal (30 mg/L) and chloramphenicol (250 mg/L). After shaking of 5g of the gathered soil from the plant root area in 45 mL of sterilized distilled water, and the suitable dilutions were made [18] Then, each sterilized Petri dish received one milliliter of the final dilution (1/1000 v/v) of the rhizosphere soil suspension, which dilution was used for the inoculation process was it 10^{-3} which was then overlaid with sterilized melted cooled PDA media. The plates were incubated for 7 days at 28±2 °C. The growing fungi were counted, isolated, and identified on PDA plates. Colony forming units (CFU) per g of rhizosphere soil were calculated [19].

Isolation of fungi from rhizoplane

In order to isolate rhizoplane fungi, the plant roots were dislodged from the adhering soil and washed several times with sterilized distilled water and dried using sterilized filter papers [18]. Then the plant roots were sliced into equal segments (about 1 cm), and four of them were placed on the surface of the Potato Dextrose Agar medium in each plate. The plates were incubated for 7 days at 28±2 °C. In order to count the colony forming units (CFU) per 12 segments of fresh roots, the growing fungus were identified,counted, and purified [20].

Isolation of fungi from phyllosphere

Plant leaves were randomly collected for phyllospheric fungi isolation. One gram of plant leaves was added to 99 mL of sterilized distilled water. After shaking, 1 mL added to 9 mL to reach the final dilution (1/1000 v/v) then one ml of the final dilution was transferred to each sterilized Petri-dish and covered with sterilized melted cooled medium. The plates were incubated for 7 days at 28 ±2 °C. Finally the growing fungi were counted, isolated, and identified and the colony forming units (CFU) per g of fresh leaves were counted [19].

Isolation of fungi from phylloplane

The previous segments of plant leaves were subjected to a series of washing with sterilized distilled water, and then dried thoroughly using sterilized filter paper; cut into equal segments (approximately 1 cm) and four of them were placed on the surface of PDA medium in each plate. The plates were incubated at 28 ± 2 °C for 7 days. The developing fungi were identified, counted, and calculated as colony forming units (CFU) per 12 segments of fresh roots [20, 21].

Identification and biodiversity of fungal isolates

The growing fungal isolates were purified on PDA and identified morphologically using macroscopic and microscopic features [22, 23].

Diversity of fungal community recovered from the collected samples was calculated including fungal taxa (s) which indicates the number of isolated fungal species from plant, dominance (d) determining the dominance of fungal taxa in specific sample; simpson index illustrates the evenness of the fungal community and Shannon index is diversity index estimates the number of fungal individuals taking in account the number of fungal taxa. Whereas, Principal component analysis (PCA) was assayed to describe the possible associations between fungal communities and the collected plants; whereas, cluster analysis was used to classify the isolated fungal into varied fungal groups. using PAleontological STatistics (PAST), Version 3.25, USA). The fungal biodiversity, Principal component analysis (PCA) and Cluster analysis were analyzed using PAleontological STatistics (PAST) program, Version 3.25, USA).

Screening for pigment production by fungal isolates

Thirty fungal isolates were selected to screen their ability for production of fungal pigments. The fungal isolates were grown on Sabouraud liquid medium supplemented with 250 mg/L chloramphenicol. The fungal cultures were incubated at 28±2 °C on an orbital shaker at 125 rpm for a period of 7 days. After 7 days of incubation, a significant amount of pigment was obtained in the flasks, since the pigments produced by the fungal isolates are water soluble; they were directly obtained in the broth. The fungal biomass was filtered using normal filter paper and the supernatant was collected and preserved in dark brown vials for characterization of fungal pigments [24-27].

Assay and characterization for fungal pigments

Assay of fungal pigments were performed by two methods; i) the collected fungal pigments were subjected to wavelength scan from 200 nm to 700 nm using UV scanning (Thermo Scientific Evolution 300) at Chemistry Department, Faculty of Sciences, Assiut university. Then, ii) concentration of the fungal pigments obtained was measured by assaying the absorbance of fungal pigments at each fungal isolate specific wavelength (obtained peak from UV scanning) using UV-120 UV-Vis spectrophotometer (Botany and Microbiology Department, Faculty of Sciences, Assiut University). Control tubes were prepared from un-inoculated Sabouraud's Dextrose broth. The concentration of fungal pigments was detected according to Beer-Lambert law: A= a.b.c (A = absorbance of pigment, a = constant, b = path length of the beam through the sample, c = concentration of pigment) [9, 12, 28-30].

RESULTS AND DISCUSSION

Isolation of rhizosphere fungi

Data in Table (1) showed that 9 genera including 18 fungal species were isolated from rhizosphere of the collected plants. Whereas, 10 fungal species belonging to 6 genera were isolated from *Medicago sativa*, 10 species belonging to 6 genera were recovered from *Triticum aestivum*, 5 genera including 10 fungal species were isolated from *Zea mays* and 10 fungal species related to 6 genera were isolated from *Vicia faba*. *Aspergillus* was the most common genus (recovered from all plant samples) comprising 50.97 % of total rhizosphere fungi on PDA medium followed by *Rhizopus stolonifer* which was recovered in moderate occurrence and the total count recorded 11.65 % of total rhizosphere fungi. *Penicillium* and *Fusarium* were recorded in low occurrence and the total count recorded 13.11% and 8.25% of total rhizosphere fungi respectively. *Alternaria, Scopulariopsis, Emericella, Eurotium* and *Chaetomium* were recorded in rare frequencies and the total count estimated 4.85%, 4.85%, 3.39%, 1.45%, 1.45%, respectively of total rhizosphere fungi. *Aspergillus* was represented by six species, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus oryzae* which were detected moderately and the total count match 9.71%, 7.28%, 10.68% and 10.19%, respectively of total rhizosphere fungi. *Aspergillus terreus* was recorded in low occurrence but it comprises 11.17% of total rhizosphere fungi whereas; *A. tamarii* was recorded in rare occurrence and the total count was 1.94% of total rhizosphere fungi. *A. flavus, A. niger* and *A. oryzae* were the most dominant species isolated from *Medicago sativa* but *A. flavus* only was the most common species isolated from *Triticum aestivum* followed by *A. fumigatus, A. oryzae* and *R. stolonifer*. In *Zea mays* plants, *Aspergillus* and *Penicillium* were the most common genera followed by *Fusarium*. *A. niger* and *P. purpurogenum* were the most prevalent species recorded from *Zea mays* plants. Whereas the most dominant species isolated from *Vicia faba* were *A. niger*, *A. terreus* and *E. nidulans* (Table 1).

Isolation of rhizoplane fungi

Eight fungal genera including twenty species were isolated as rhizoplane fungi on PDA medium from collected plants (Table 2). Whereas, 13 fungal species belonging to 7 fungal genera were estimated from *Medicago sativa*, 11 species belonging to 8 genera were recorded from *Triticum aestivum* as well as 11 species belonging to 8 genera were recovered from *Zea mays* but 10 species belonging to 3 genera were recorded from *Vicia*

faba plants. *Aspergillus* was the most prevalent genus comprising 38.82 % of total rhizoplane fungi on PDA medium followed by *Fusarium, Penicillium* and *Emericella* which were recorded in moderate frequency and the total count estimated 14.11 %, 11.18 % and 8.82 %, respectively of total rhizoplane fungi. *Alternaria, Eurotium* and *Rhizopus* were recorded in low frequencies and the total count comprising 9.41%, 5.59% and 5.59%, respectively of total rhizoplane fungi whereas; *Epicoccum* was recorded in rare frequency matching 4.12% of total rhizoplane fungi. *Aspergillus* was represented by seven species, *Aspergillus flavus* and *Aspergillus terreus* were recorded moderately, and the total count of them comprising 7.35% of total rhizoplane fungi. *Aspergillus niger* and *Aspergillus tamarii* were recorded in low occurrence but the total counts were 13.53% and 2.35%, respectively of total rhizoplane fungi. *Aspergillus clavatus, Aspergillus fumigatus* and *Aspergillus oryzae* were recorded in rare occurrence and the total count match 2.65%, 2.94% and 2.65%, respectively of total rhizoplane fungi. From *Medicago sativa* plants, *Aspergillus* and *Penicillium* were the most common genera (recovered in all plant samples) but *Penicillium purpurogenum* was the most common species. In the other hand, *Fusarium* the most common genus isolated from *Triticum aestivum* plants. *Emericella nidulans, Fusarium solani, Aspergillus flavus* and *Penicillium purpurogenum* were the most prevalent species. *Aspergillus* was the most common genus isolated from *Zea mays* also *Alternaria alternata, Aspergillus terreus, Emericella nidulans* and *Penicillium purpurogenum* were the most common species. In *Vicia faba,* the most common genera were *Aspergillus* and *Penicillium*, whereas the most common species was *Aspergillus terreus* (Table 2). According to our findings, *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, and *Penicillium chrysogenum* were isolated as rhizosphere fungi from Egyptian plants [31], and *Aspergillus ochraceus*, *Fusarium solani*, and *Penicillium purpurogenum* were recovered from rhizosphere and rhizoplane samples of cultivated plants from Assiut Governorate [32], While the most frequent fungi found in Egyptian maize rhizosphere samples were *Aspergillus flavus*, *Aspergillus terreus*, *Fusarium oxysporum*, *Fusarium nygamia*, and *Fusarium solani*, whereas, the most common fungi recorded for maize rhizoplane samples included *Fusarium* and *Cochliobolus* [18]. *Aspergillus flavus*, *Aspergillus niger*, and *Rhizopus stolonifer* were frequent species in an earlier investigation that isolated 14 species from the faba bean rhizosphere and rhizoplane [33].

Isolation of phyllosphere fungi

The obtained results in Table (3) revealed that, 20 fungal species belonging to 7 genera were recovered from phyllosphere of collected plants. Whereas, 6 fungal genera including 10 species were estimated from *Medicago sativa* and 9 fungal species belonging to 6 genera were isolated from *Triticum aestivum* plants. As well as, four genera including 11 fungal species were recovered from *Zea mays* and 4 genera including 10 species were isolated from *Vicia faba*. *Aspergillus* was the most prevalent genus comprising 45.92 % of the total fungi followed by *Penicillium, Fusarium* and *Alternaria* contributing 22.96%, 12.24% and 8.16%, respectively of total phyllosphere fungi on PDA medium. Whereas *Rhizopus* was recorded in low occurrence and the total count match 6.63% of total phyllosphere fungi, *Emericella* and *Eurotium* were recorded in rare occurrence and the total count estimated 2.55%, 1.53% of total phyllosphere fungi, respectively. *Aspergillus* was represented by seven species, from which *A. flavus* and *A. niger* were detected moderately and the total count comprise 13.27%, 10.2% of total

phyllosphere fungi, respectively. Whereas, *A. fumigatus, Aspergillus oryzae* and *Aspergillus terreus* were recorded in low occurrence and the total count comprise 5.61%, 6.12% and 6.63%, respectively of total phyllosphere fungi. In the other hand, *A. clavatus* and *A. tamarii* were recorded in rare occurrence (the total count of them comprising 2.04% of total phyllosphere fungi). In *Medicago sativa, Aspergillus* was the most common genus (recovered in all plant samples) followed by *Penicillium*. In *Triticum aestivum* plants, the most common genera were *Aspergillus, Alternaria* and *Rhizopus* followed by *Penicillium* and *Fusarium. Aspergillus terreus* was the most prevalent species followed by *Fusarium solani.* In *Zea mays* plants, *Aspergillus* and *Fusarium* were the most common genera (recovered in all plant samples) followed by *Penicillium*. As well, *Aspergillus flavus* and *Aspergillus tamarii* were the most common species. In *Vicia faba* plants, *Aspergillus* and *Penicillium* were the most common genera (recovered from all plant samples) followed by *Alternaria*. As, *Alternaria alternata, Aspergillus fumigatus, Aspergillus oryzae, Penicillium chrysogenum and Penicillium glabrum were* the most prevalent species (Table 3). It was reported that, the most prevalent genus isolated from all phyllosphere samples of faba bean as *Aspergillus*, which accounted for 50.87% of the total number of phyllosphere fungal species. *Rhizopus stolonifer* came in second, contributing 19.56% of the total number of phyllosphere fungi [34]. The genus *Penicillium* has been described from the phyllosphere of other plants, including wheat [35], which is consistent with our results. In a different study, the phyllosphere of maize plants contained 19 species from 10 genera. The most prevalent genus, *Aspergillus*, estimating 16.2% of all fungi and was recorded in all Samples with a high frequency of occurrence. Furthermore, the second most common fungus, *Penicillium*, was found in all samples and accounted for 22.6% of the total fungi recovered from phyllosphere of maize plants After *Penicillium, Fusarium* came in third place and was noted as moderate prevalent, producing 28.2% of all fungi isolated from phyllosphere of maize plants [18].

Isolation of phylloplane fungi

In the current study, eight fungal genera including twenty-two species were isolated as phylloplane fungi on PDA medium (Table 4). Fifteen fungal species belonging to 7 genera were recovered from *Medicago sativa*, 10 fungal species belonging to 6 genera were isolated from *Triticum aestivum*. As well, 12 species belonging to 6 genera were isolated from *Zea mays* plants and 10 fungal species belonging to 7 genera were isolated from *Vicia faba* plants. *Aspergillus* was the most common genus comprising 46.28% followed by *Penicillium, Emericella* and *Alternaria* were recorded in moderate occurrence and the total count match 11.15%, 10.13% and 9.46% of total phylloplane fungi, respectively on PDA medium. *Epicoccum*, *Eurotium, Rhizopus* and *Fusarium* was recovered in low occurrence and the total count contribute 7.43%, 5.41%, 5.74% and 4.39% of total phylloplane fungi, respectively. *Aspergillus* was represented by seven species, *A. flavus* and *A. niger* which were detected moderately and the total count comprise 13.27%, 10.2% of total phyllosphere fungi, respectively. *Aspergillus oryzae* was detected in moderate occurrence and the total count estimated 15.54% of total phylloplane fungi but other species were detected in low and rare occurrence. In *Medicago sativa, Aspergillus* and *Alternaria* were the most common genera and *A. niger* and *A. flavus* were the most prevalent species. In *Triticum aestivum* plants, the most common genera were *Aspergillus* and *Penicillium* followed by *Alternaria* and *Epicoccum. Aspergillus ochraceus, Aspergillus oryzae, Penicillium citrinum* and

Penicillium purpurogenum were the most common species recovered from *Triticum aestivum* plants. Whereas, in *Zea mays* plants, *Aspergillus, Emericella* and *penicillium* were the most common genera as well, *A. flavus* and *A. ochraceus* and *A. oryzae* were the most prevalent species. In *Vicia faba, Aspergillus* and *Emericella* were the most common genera followed by *Alternaria, Eurotium* and *Penicillium* whereas *Alternaria alternata, A. clavatus, A. oryzae* and *Penicillium decumbens* were the most common species (Table 4).

An early investigation stated that, filamentous fungi on the surface of plant leaves, represents a diverse terrestrial environment. *Aspergillus niger, Candida* sp., *Cladosporium herbarum*, *Fusarium* sp., *Epicoccum nigrum*, *Penicillum* sp., and *Alternaria alternata* were among the fungi isolated from the phylloplane of a healthy *Sapindus mukorossi* leaf during the study [36]. In a different study, the phylloplane of maize plants collected from Assiut Governorate revealed twenty-one species and one variety belonging to twelve genera. The most common genus, *Aspergillus*, made up 40.6% of all the fungi and was found in high frequency of occurrence. Additionally, *Penicillium* was found in moderate occurrence, accounting for 6% of all fungus. *Alternaria alternata* was isolated with a low frequency of occurrence recording 3.3% of all fungi [18].

Biodiversity analysis of isolated fungi from collected plants

Data presented in Table (5) showed that the highest fungal dominance was recorded for *Medicago sativa* plants followed by *Vicia faba* whereas the lowest dominance was recorded for *Triticum aestivum* plants. The highest Simpson (0.9414) and Shannon (2.969) diversity indexes was recorded for *Triticum aestivum* plant samples. The highest fungal evenness was estimated for *Vicia faba* plants recording 0.9155 and the lowest fungal evenness (0.7986) was recorded for *Medicago sativa* plants. The principal component analysis revealed that the high abundance of *Aspergillus fumigatus, Aspergillus terreus, Aspergillus oryzae, Aspergillus niger* and *Rhizopus stolonifer* was correlated positively with *Vicia faba* samples (Fig. 1). The occurrence of fungal species *Aspergillus tamari*, *Eurotium chevalieri*, *Fusarium solani*, *Alternaria alternata* and *Aspergillus flavus* were closely related to *Zea mays*, *Medicago sativa,* and *Triticum aestivum* (Fig. 1). The cluster analysis revealed that, there are 4 distinct fungal groups, the first group included *Penicillium glabrum*, *Penicillium funiculosum* and *Aspergillus clavatus*. Whereas the second group is the largest group included *Penicillium chrysogenum*, *Scopulariopsis brumptii*, *Penicillium decumbens*, *Fusarium oxysporum*, *Penicillium digitatum*, *Penicillium expansum, Chaetomium* sp., *Penicillium duclauxi*, *Aspergillus wentii*, *Aspergillus ustus*, *Aspergillus parasiticus*, *Eurotium chevalieri*, *Penicillium citrinum*, *Aspergillus tamari*, *Epicocum nigrum*, *Fusarium verticillioides*. The third fungal group contained *Aspergillus ochraceus*, *Rhizopus stolonifer* and *Alternaria alternata* and the fourth group represented by *Fusarium solani, Aspergillus terreus, Aspergillus fumigatus, Emericella nidulans, Aspergillus oryzae, Aspergillus niger* and *Aspergillus flavus*. So, the cluster analysis showed that the different plant types showed varied fungal community structures (Fig. 2).

Assay and characterization of pigment production by fungi

Thirty fungal isolates recovered from different parts of plants (*Medicago sativa, Triticum aestivum, Zea mays, Vicia faba),* were tested for their abilities to produce fungal pigments on Sabouraud's Dextrose broth (Table 6). The results showed that 19 fungal isolates (out

of 30 tested fungal isolates) exhibited various degrees of pigment production on liquid medium. The obtained results of tested fungal pigments using UV-Visible spectroscopy revealed the presence of different compounds showing various peaks based on absorption maxima. As well as, absorption peaks of pigments recorded from all fungal isolates were found in the range of 220 nm and 490 nm. *Alternaria alternata* (Alt1), *Alternaria alternata* (Alt2) showed absorption peaks at wavelength 230 nm and 284 nm. (Fig 3C) Likewise, *Aspergillus flavus* (Af2) (Fig 3D) and *Aspergillus niger* (An1) showed peaks at wavelength 270 nm to 238 nm while *Aspergillus ochraceus* (Ao1) and *Epicoccum nigrum* (En) showed peaks at 264, different species of *Aspergillus terreus* (At1- At6) showed peaks between 262 to 282 nm (Fig 3B). *Emericella nidulans* (Em1) and *Emericella nidulans* (Em2) showed absorption peaks at wavelength 286 and 228 respectively. *Erotium chevalieri* (Eu) and *Penicillium chrysogenum* (Pch) showed absorption peaks at wavelength 262 as well, *Penicillium citrinum* (Pc) showed absorption peak at wavelength 268 (Fig 3F). In case of *Penicillium purpurogenum* (PP1) showed peak at 490 nm. Interestingly, only *Penicillium purpurogenum* (PP2) gave two peaks; one of them in UV region at wavelength 220 and second peak in visible light region at wavelength 490 (Fig 3E). Figure (3) showing some representative results obtained from UV scanning of fungal pigments. Fungi are considered natural sources for the production of secondary metabolites, e.g., pigments [37-41]. It was reported that, pure cultures of filamentous fungi e.g. *A. alternata*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. sydowii*, *A. tamari*, *A. terreus*, *A. tubingensis*, *E. nigrum*, *Exophiala pisciphila*, *Magnapothe grisea*, *Ochroconis anomala*, *O. lascauxensis*, *Penicillium marneffei*, *Phyllosticta capitalensis* and *Stachybotry chartarum* could produce melanin after being cultured in both solid and liquid media [39, 42-46]. Furthermore, *Talaromyces australis*, *Penicillium murcianum*, *Talaromyces* sp., *Trichoderma spirale*, and *Fusarium oxysporum*, were recorded for various natural pigments production when grown in liquid media.[47] The produced pigments by filamentous fungi are secondary metabolites whose production usually commences late in the growth of the microorganisms, especially when entering the stationary phase [48].

Determination of fungal pigment concentration

The obtained data (Table 7) revealed that, out of nineteen fungal isolates, only seven pigment producing fungal isolates were considered as highly producers (conc. > 3g/L) and eight fungal isolates exhibited moderate pigment production (1-3g/L), and 4 isolates achieved low pigment production (conc. < 1g/l). *Penicillium purpurogenum* (PP2) showed the highest pigment production on Sabouraud's Dextrose broth yielding 5.156 g/L. followed by *Alternaria alternata* (Alt1), *Alternaria alternata* (Alt2), *Aspergillus terreus* (At1), *Aspergillus terreus* (At5), *Emericella nidulans* (Em1) and *Penicillium purpurogenum* (PP1) yielding 4.164, 4.611, 3.675, 3.303, 4.931 and 5.031 g/L, respectively. The second group contains 8 fungal isolates that considered as moderate producers of pigment recording fungal pigment concentration 1-3 g/L. These isolates were *Aspergillus terreus* (At2), *Aspergillus terreus* (At3), *Aspergillus terreus* (At4), *Aspergillus terreus* (At6), *Emericella nidulans* (Em2), *Epicoccum nigrum* (En), *Eurotium chevalieri* (Eu) and *Penicillium chrysogenum* (Pch) recording 1.758, 2.586, 1.809, 1.498, 2.673, 1.187, 1.959, 1.682 g/L, respectively. The remaining fungal isolates (4 out of 19) showed low production abilities of pigment (conc.<1g/L) including *Aspergillus flavus* (Af2), *Aspergillus niger* (An1), *Aspergillus ochraceus* (Ao1) and *Penicillium citrinum*

(Pc) estimating 0.467, 0.256, 0.280 and 0.735 g/L, respectively. Furthermore, the range of pigments produced by the isolated fungi varied from red, reddish brown, orange, black, brown and yellow (Fig. 4). Interestingly, it was stated that, filamentous fungi have ability to produce a range of pigments that varied from yellow, to red, orange and purple [39, 47, 49]. These pigments are produced as extracellular metabolites [50], and are preferred to study and investigate because of their solubility in culture media, and consequently, ease and low cost of downstream processing . The obtained results in this study were similar to those of previously reported which found that the highest level of absorbance of the fungal pigment melanin produced by various fungi were located in the UV region ranging from 200−300 nm and decreased toward the visible region [11, 39, 42, 43, 45, 46, 51-53]. A previous study stated that, *Epicoccum nigrum* was a potent pigment producing fungus recording 8.19 g/L [48] Furthermore, *Emericella nidulans* showed pigment production with the yield of 0.700– 0.800 g/L and *Penicillium purpurogenum* produced 20 mg /mL of pigment concentration [54, 55]. It was reported that, various *Eurotium* species of including *E. amstelodami*, *E. chevalieri* and *E. herbariorum* have been found to produce the yellow pigments [56]. Furthermore, *Alternaria alternata* was recorded to produce reddish brown pigments with optical density 2.013 [57] also, *Aspergillus niger* produced 2.19 mg/mL of pigment, *Aspergillus flavus produced* 1.43 mg/mL and *Aspergillus terreus* 0.22 mg/ mL [42].

Table 1 : Total count , percentage of total count TC% , number of cases of isolation (out of 16 samples) and occurrence of fungi isolated from rhizosphere plants (four samples from each plant) on potato dextrose agar medium (PDA).

samples / Fungal species	*Medicago sativa*			*Triticum aestivum*			*Zea mays*			*Vicia faba*			Total			
	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	OR
Alternaria alternata	0	0	0	3000	7.32	2	7000	13.46	2	0	0	0	10000	4.85	4	R
Aspergillus	39000	68.42	4	25000	60.98	4	20000	38.46	4	21000	37.5	4	105000	50.97	16	H
A. flavus	6000	10.53	3	9000	21.95	4	5000	9.62	2	0	0	0	20000	9.71	9	M
A. fumigatus	3000	5.26	2	4000	9.76	3	3000	5.77	2	5000	8.93	2	15000	7.28	9	M
A. niger	5000	8.77	3	4000	9.76	2	7000	13.46	3	6000	10.71	3	22000	10.68	11	M
A. oryzae	9000	15.79	3	4000	9.76	3	5000	9.62	2	3000	5.36	1	21000	10.19	9	M
A. tamari	0	0	0	4000	9.76	2	0	0	0	0	0	0	4000	1.94	2	R
A. terreus	16000	28.07	2	0	0	0	0	0	0	7000	12.5	3	23000	11.17	5	L

Chaetomium sp.	**3000**	**5.26**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3000**	**1.46**	**1**	**R**
Emericella nidulans	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7000**	**12.5**	**3**	**7000**	**3.40**	**3**	**R**
Eurotium	**3000**	**5.26**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3000**	**1.46**	**2**	**R**
Fusarium	**6000**	**10.53**	**2**	**1000**	**2.44**	**1**	**7000**	**13.46**	**3**	**3000**	**5.36**	**1**	**17000**	**8.25**	**7**	**L**
F. solani	**6000**	**10.53**	**2**	**1000**	**2.44**	**1**	**4000**	**7.69**	**2**	**3000**	**5.36**	**1**	**14000**	**6.80**	**6**	**L**
F. verticillioides	**0**	**0**	**0**	**0**	**0**	**0**	**3000**	**5.77**	**2**	**0**	**0**	**0**	**3000**	**1.46**	**2**	**R**
Penicillium	**2000**	**3.51**	**1**	**2000**	**4.88**	**2**	**12000**	**23.08**	**4**	**11000**	**19.64**	**2**	**27000**	**13.11**	**8**	**L**
P. chrysogenum	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**8000**	**14.29**	**2**	**8000**	**3.88**	**2**	**R**
P. digitatum	**2000**	**3.51**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2000**	**0.97**	**1**	**R**
P. funiculosum	**0**	**0**	**0**	**2000**	**4.88**	**2**	**4000**	**7.69**	**2**	**3000**	**5.36**	**2**	**9000**	**4.37**	**6**	**L**
P. purpurogenum	**0**	**0**	**0**	**0**	**0**	**0**	**8000**	**15.38**	**3**	**0**	**0**	**0**	**8000**	**3.88**	**3**	**R**
Rhizopus stolonifer	**4000**	**7.02**	**2**	**9000**	**21.95**	**3**	**6000**	**11.54**	**2**	**5000**	**8.93**	**2**	**24000**	**11.65**	**9**	**M**
Scopulariopsis brumptii	**0**	**0**	**0**	**1000**	**2.439024**	**1**	**0**	**0**	**0**	**9000**	**16.07**	**2**	**10000**	**4.85**	**3**	**R**
Total count	**57000**			**41000**			**52000**			**56000**			**206000**			
No. of genera	**6**			**6**			**5**			**6**			**10**			
No. of species	**10**			**10**			**10**			**10**			**18**			

TC = Total count, TC% = percentage of total count, NCI = Number of cases of isolation, OR = Occurrence remark; H= High occurrence, more than 12; M=Moderate occurrence 9-12; L=Low occurrence 5-8; R=Rare occurrence, less than 5

Table 2: Total count, percentage of total count, number of cases of isolation (out of 16 samples) and occurrence of fungi isolated from rhizoplane of plants (four samples from each plant) on potato dextrose agar medium (PDA).

samples / Fungal species	*Medicago sativa*			*Triticum aestivum*			*Zea mays*			*Vicia faba*			total			
	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	OR
Alternaria alternata	48	15.84158	2	0	0	0	48	17.204301	3	0	0	0	96	9.411765	5	L
Aspergillus	132	43.56435	4	39	20.3125	3	51	18.279569	4	174	70.73171	4	396	38.82353	15	H
A. clavatus	0	0	0	6	3.125	1	0	0	0	21	8.536585	3	27	2.647059	4	R
A. flavus	18	5.940594	2	21	10.9375	3	24	8.6021505	2	12	4.878048	2	75	7.352941	9	M
A. fumigatus	15	4.950495	2	0	0	0	0	0	0	15	6.09756	2	30	2.941176	4	R
A. niger	54	17.82178	2	12	6.25	2	12	4.301075	1	60	24.39024	3	138	13.52941	8	L
A. oryzae	15	4.950495	2	0	0	0	0	0	0	12	4.878048	2	27	2.647058	4	R
A. tamari	9	2.970297	2	0	0	0	0	0	0	15	6.09756	3	24	2.352941	5	L
A. terreus	21	6.930693	3	0	0	0	15	5.376344	3	39	15.85365	4	75	7.352941	10	M
Emericella nidulans	18	5.940594	3	51	26.5625	3	21	7.5268817	3	0	0	0	90	8.823529	9	M
Epicocum nigrum	0	0	0	9	4.6875	2	33	11.827956	2	0	0	0	42	4.117647	4	R
Eurotium chevalieri	6	1.980198	1	9	4.6875	2	42	15.053763	2	0	0	0	57	5.588235	5	L
Fusarium	42	13.86138	3	51	26.5625	4	51	18.279569	2	0	0	0	144	14.11764	9	M
F. oxxysporum	0	0	0	15	7.8125	2	0	0	0	0	0	0	15	1.470588	2	R
F. solani	42	13.86138	3	36	18.75	3	51	18.279569	2	0	0	0	129	12.64705	8	L
Penicillium	48	15.84158	4	9	4.6875	2	12	4.3010752	2	45	18.29268	4	114	11.17647	12	M
P. chrysogenum	0	0	0	9	4.6875	2	0	0	0	0	0	0	9	0.882353	2	R
P. citrinum	0	0	0	0	0	0	12	4.301075	2	15	6.09756	3	27	2.647059	5	L

Table 2: Continued

P. duclauxi	**21**	**6.930693**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**2.058824**	**2**	**R**
P. funiculosum	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**12**	**4.878048**	**3**	**12**	**1.176471**	**3**	**R**
P. glabrum	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**	**7.317073**	**3**	**18**	**1.764706**	**3**	**R**
P. purpurogenum	**27**	**8.910891**	**4**	**12**	**6.25**	**3**	**12**	**4.301075**	**3**	**0**	**0**	**0**	**51**	**5**	**10**	**M**
Rhizopus stolonifer	**9**	**2.970297**	**1**	**12**	**6.25**	**2**	**9**	**3.225806**	**1**	**27**	**10.9756**	**2**	**57**	**5.588235**	**6**	**L**
Total count	**303**	**100**		**192**	**100**		**279**	**100**		**246**	**100**		**1020**	**100**		
No. of genera	**7**			**8**			**8**			**3**			**8**			
No. of species	**13**			**11**			**11**			**10**			**20**			

TC = Total count, TC% = percentage of total count, NCI = number of cases of isolation, OR = occurrence remark; H= High occurrence, more than 12; M=Moderate occurrence 9-12; L=Low occurrence 5-8; R=Rare occurrence, less than 5

Table 3: Total count, percentage of total count, number of cases of isolation (out of 16 samples) and occurrence of fungi isolated from phyllosphere of plants (four samples from each plant) on potato dextrose agar medium (PDA).

Samples / Fungal species	*Medicago sativa*			*Triticum aestivum*			*Zea mays*			*Vicia faba*			Total			
	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	OR
Alternaria alternata	5000	13.51	2	7000	14.89	4	0	0	0	4000	6.90	3	16000	8.16	9	M
Aspergillus	17000	45.95	4	13000	27.66	4	29000	53.70	4	31000	53.44	4	90000	45.92	16	H
A. clavatus	0	0	0	1000	2.13	1	0	0	0	3000	5.17	2	4000	2.04	3	R
A. flavus	6000	16.22	2	0	0	0	13000	24.07	3	7000	12.07	4	26000	13.27	9	M
A. fumigatus	3000	8.11	1	2000	4.26	2	0	0	0	6000	10.34	3	11000	5.61	6	L
A. niger	6000	16.22	3	3000	6.38	2	8000	14.81	2	3000	5.17	2	20000	10.20	9	M
A. tamari	0	0	0	0	0	0	4000	7.41	3	0	0	0	4000	2.04	3	R

A. terreus	0	0	0	7000	14.89	4	0	0	0	6000	10.34	3	13000	6.63	7	L
Emericella nidulans	3000	8.11	1	2000	4.26	2	0	0	0	0	0	0	5000	2.55	3	R
Eurotium sp.	0	0	0	0	0	0	3000	5.56	2	0	0	0	3000	1.53	2	R
Fusarium	3000	8.11	1	8000	17.02	3	8000	14.81	4	5000	8.62	2	24000	12.24	10	M
F. oxxysporum	0	0	0	0	0	0	2000	3.70	1	0	0	0	2000	1.02	1	R
F. solani	3000	8.11	1	8000	17.02	3	2000	3.70	1	5000	8.62	2	18000	9.18	7	L
F. verticillioides	0	0	0	0	0	0	4000	7.41	2	0	0	0	4000	2.04	2	R
Penicillium	7000	18.92	3	6000	12.77	3	14000	25.93	3	18000	31.03	4	45000	22.96	13	H
P. chrysogenum	0	0	0	3000	6.38	2	0	0	0	13000	22.41	3	16000	8.16	5	L
P. citrinum	3000	8.11	2	0	0	0	0	0	0	0	0	0	3000	1.53	2	R
P. digitatum	4000	10.81	2	0	0	0	0	0	0	0	0	0	4000	2.04	2	R
P.funiculosum	0	0	0	0	0	0	4000	7.41	2	0	0	0	4000	2.04	2	R
P. glabrum	0	0	0	3000	6.38	2	5000	9.26	2	5000	8.62	3	13000	6.63	7	L
P. purpurogenum	0	0	0	0	0	0	5000	9.26	2	0	0	0	5000	2.55	2	R
Rhizopus stolonifera	2000	5.41	1	11000	23.40	4	0	0	0	0	0	0	13000	6.63	5	L
Total count	3000			47000			54000			58000			196000			
No. of genera	6			6			4			4			7			
No. of species	10			9			11			10			20			

TC = Total count, TC% = percentage of total count, NCI = number of cases of isolation, OR = occurrence remark; H= High occurrence, more than 12; M=Moderate occurrence, 9-12; L=Low occurrence, 5-8; R=Rare occurrence, less than 5

Table 4: Total count, percentage of total count, number of cases of isolation (out of 16 samples) and occurrence of fungi isolated from phylloplane of plants (four samples from each plant) on potato dextrose agar medium (PDA).

Samples / Fungal species	*Medicago sativa*			*Triticum aestivum*			*Zea mays*			*Vicia faba*			total			
	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	TC	TC %	NCI	OR
Alternaria alternata	30	13.51	4	15	9.62	3	12	4.04	2	27	12.68	3	84	9.46	12	M
Aspergillus	123	55.41	4	57	36.54	4	141	47.47	4	90	42.25	4	411	46.28	16	H
A. clavatus	0	0	0	0	0	0	0	0	0	18	8.45	3	18	2.03	3	R
A. flavus	33	14.86	3	0	0	0	15	5.05	3	24	11.27	2	72	8.11	8	L
A. fumigatus	12	5.41	2	0	0	0	0	0	0	0	0	0	12	1.35	2	R
A. niger	24	10.81	3	0	0	0	30	10.10	2	24	11.27	2	78	8.78	7	L
A. ochraceus	0	0	0	15	9.62	3	15	5.05	3	0	0	0	30	3.38	6	L
A. oryzae	12	5.41	2	33	21.15	3	69	23.23	3	24	11.27	3	138	15.54	11	M
A. parasiticus	9	4.05	2	0	0	0	0	0	0	0	0	0	9	1.01	2	R
A. tamarii	0	0	0	9	5.77	2	6	2.02	2	0	0	0	15	1.69	4	R
A. terreus	15	6.76	2	0	0	0	6	2.02	1	0	0	0	21	2.36	3	R
A. ustus	9	4.05	2	0	0	0	0	0	0	0	0	0	9	1.01	2	R
A. wentii	9	4.05	2	0	0	0	0	0	0	0	0	0	9	1.01	2	R
Emericella nidulans	15	6.76	2	0	0	0	51	17.17	4	24	11.27	4	90	10.12	10	M
Epicoccum nigrum	0	0	0	15	9.62	3	51	17.17	2	0	0	0	66	7.43	5	L
Eurotium sp.	15	6.76	2	0	0	0	9	3.03	2	24	11.27	3	48	5.41	7	L
Fusarium	21	9.46	2	9	5.77	2	0	0	0	9	4.23	2	39	4.39	6	L
F. solani	9	4.05	1	9	5.77	2	0	0	0	9	4.23	2	27	3.04	5	L
F. verticilloides	12	5.41	1	0	0	0	0	0	0	0	0	0	12	1.35	1	R
Penicillium	3	1.35	1	48	30.77	4	33	11.11	4	15	7.04	3	99	11.15	12	M
P. citrinum	0	0	0	18	11.54	3	9	3.03	2	0	0	0	27	3.04	5	L

Table 4: Continued

P. decumbens	0	0	0	9	5.77	2	0	0	0	15	7.04	3	24	2.70	5	L
P. expansum	3	1.35	1	0	0	0	0	0	0	0	0	0	3	0.34	1	R
P. purpurigenum	0	0	0	21	13.46	3	24	8.08	2	0	0	0	45	5.07	5	L
Rhizopus stolonifer	15	6.76	2	12	7.69	2	0	0	0	24	11.27	2	51	5.74	6	L
Total count	**222**	**100**		**156**	**100**		**297**	**100**		**213**			**888**			
No. of genera	**7**			**6**			**6**			**7**			**8**			
No. of species	**15**			**10**			**12**			**10**			**22**			

TC = Total count, TC% = percentage of total count, NCI = number of cases of isolation, OR = occurrence remark; H= High occurrence, more than 12; M=Moderate occurrence, 9-12; L=Low occurrence, 5-8; R=Rare occurrence, less than 5

Table 5: Biodiversity of fungal community recovered from collected plants.

Plants **Diversity statics**	***Medicago sativa***	***Triticum aestivum***	***Zea mays***	***Vicia faba***
Taxa (S)	**21**	**23**	**19**	**18**
Dominance (D)	**0.06855**	**0.05863**	**0.06554**	**0.06497**
Simpson(1-D)	**0.9314**	**0.9414**	**0.9345**	**0.935**
Shannon (H)	**2.82**	**2.969**	**2.82**	**2.802**
Evenness($e^{H/S}$)	**0.7986**	**0.8469**	**0.8827**	**0.9155**

Table 6: UV scanning results of tested fungal pigments

	Parameter / Fungal isolates	Wave length	Absorbance
	media	**262.00**	**1.564**
1	***Alternaria alternata* (Alt1)**	**230**	**2.585**
2	***Alternaria alternata* (Alt2)**	**284**	**1.197**
3	***Aspergillus flavus* (Af1)**	**Not Detected**	**N.D**
4	***Aspergillus flavus* (Af2)**	**270**	**2.543**
5	***Aspergillus flavus* (Af3)**	**N.D**	**N.D**
6	***Aspergillus flavus* (Af4)**	**N.D**	**N.D**
7	***Aspergillus niger* (An1)**	**238**	**2.044**
8	***Aspergillus niger* (An2)**	**N.D**	**N.D**
9	***Aspergillus niger* (An3)**	**N.D**	**N.D**
10	***Aspergillus niger* (An4)**	**N.D**	**N.D**
11	***Aspergillus niger* (An5)**	**N.D**	**N.D**
12	***Aspergillus ochraceus* (Ao1)**	**264**	**2.445**
13	***Aspergillus ochraceus* (Ao2)**	**N.D**	**N.D**
14	***Aspergillus ochraceus* (Ao3)**	**N.D**	**N.D**
15	***Aspergillus ochraceus* (Ao4)**	**N.D**	**N.D**
16	***Aspergillus ochraceus* (Ao5)**	**N.D**	**N.D**
17	***Aspergillus terreus* (At1)**	**282**	**2.561**
18	***Aspergillus terreus* (At2)**	**282**	**1.343**
19	***Aspergillus terreus* (At3)**	**262**	**1.019**
20	***Aspergillus terreus* (At4)**	**282**	**1.574**
21	***Aspergillus terreus* (At5)**	**282**	**2.58**
22	***Aspergillus terreus* (At6)**	**280**	**1.649**

Table 6: Continued

23	***Emericella nidulans* (Em1)**	**286**	**2.123**
24	***Emericella nidulans* (Em2)**	**228**	**2.390**
25	***Epicoccum nigrum* (En)**	**264**	**2.096**
26	***Erotium chevalieri* (Eu)**	**262**	**0.868**
27	***Penicillium chrysogenum* (Pch)**	**262**	**0.668**
28	***Penicillium citrinum* (Pc)**	**268**	**2.758**
29	***Penicillium purpurogenum* (PP1)**	**490**	**0.431**
30	***Penicillium purpurogenum* (PP2)**	**490**	**1.557**

Table 7: Concentrations and color of fungal pigments obtained from tested fungal isolates

Parameters / Fungal isolates	Color of pigment	Concentration (g/L)
***Alternaria alternata* (Alt1)**	**Dark brown**	**4.164372**
***Alternaria alternata* (Alt2)**	**Dark brown**	**4.610653**
***Aspergillus flavus* (Af2)**	**Yellow**	**0.467402**
***Aspergillus niger* (An1)**	**Yellow with black shade**	**0.256198**
***Aspergillus ochraceus* (Ao1)**	**Pale brown**	**0.280074**
***Aspergillus terreus* (At1)**	**Brown**	**3.674931**
***Aspergillus terreus* (At2)**	**Pale brown**	**1.757576**
***Aspergillus terreus* (At3)**	**brown**	**2.58586**
***Aspergillus terreus* (At4)**	**Light brown**	**1.809917**
***Aspergillus terreus* (At5)**	**brown**	**3.30303**
***Aspergillus terreus* (At6)**	**Reddish brown**	**1.497705**
***Emericella nidulans* (Em1)**	**Reddish brown**	**4.931129**

Emericella nidulans **(Em2)**	**Deep yellow**	**2.673093**
Epicoccum nigrum **(En)**	**orange**	**1.187328**
Erotium chevalieri **(Eu)**	**yellow**	**1.958678**
Penicillium chrysogenum **(Pch)**	**yellow**	**1.682278**
Penicillium citrinum **(Pc)**	**Yellow to pale brown**	**0.73462**
Penicillium purpurogenum **(PP1)**	**Red**	**5.03122**
Penicillium purpurogenum **(PP2)**	**Dark Red**	**5.156107**

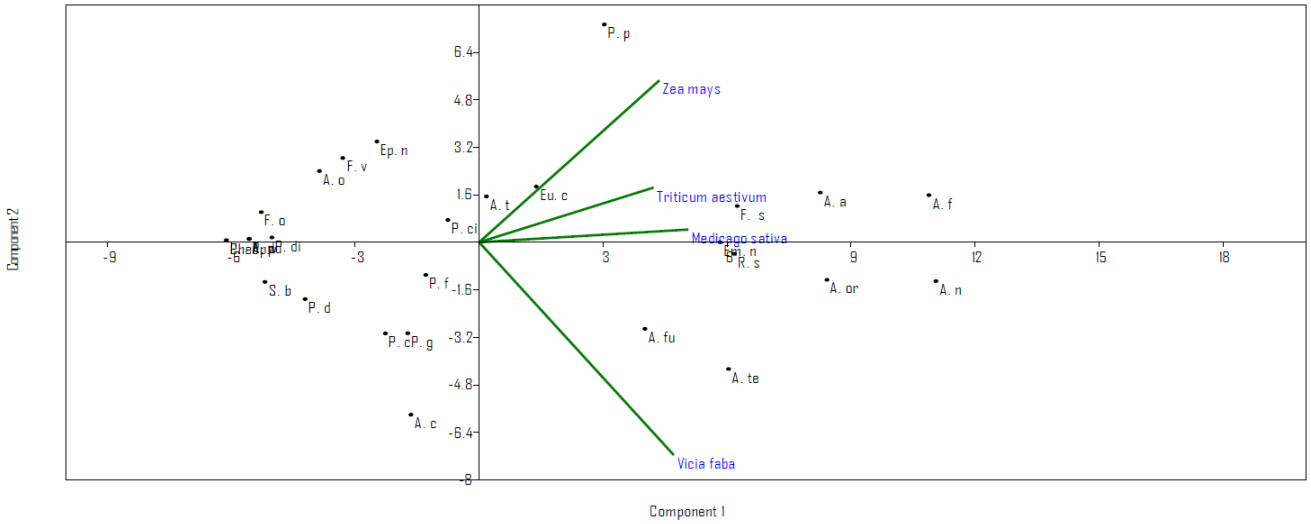


Fig. 1: Principal component analysis plot for fungal communities in correlation with different plants.

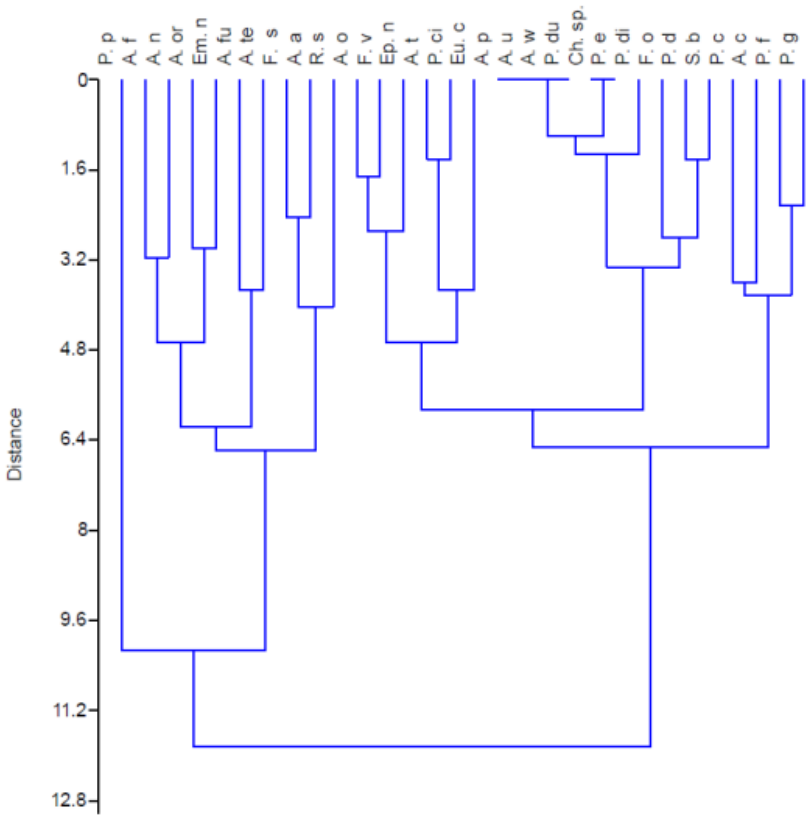
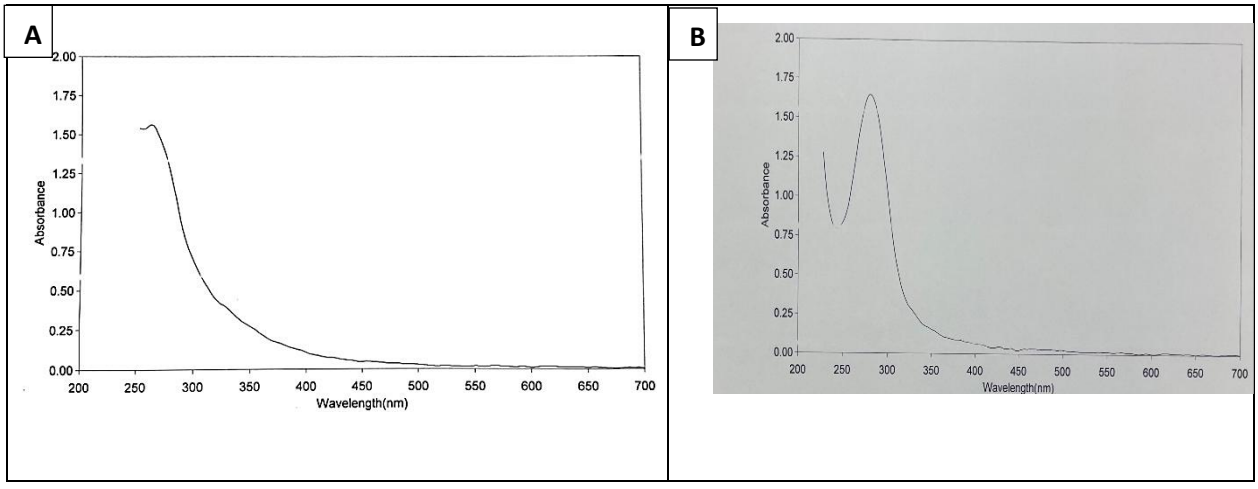


Fig. 2: Cluster analysis showing the fungal communities recovered from collected plant samples.



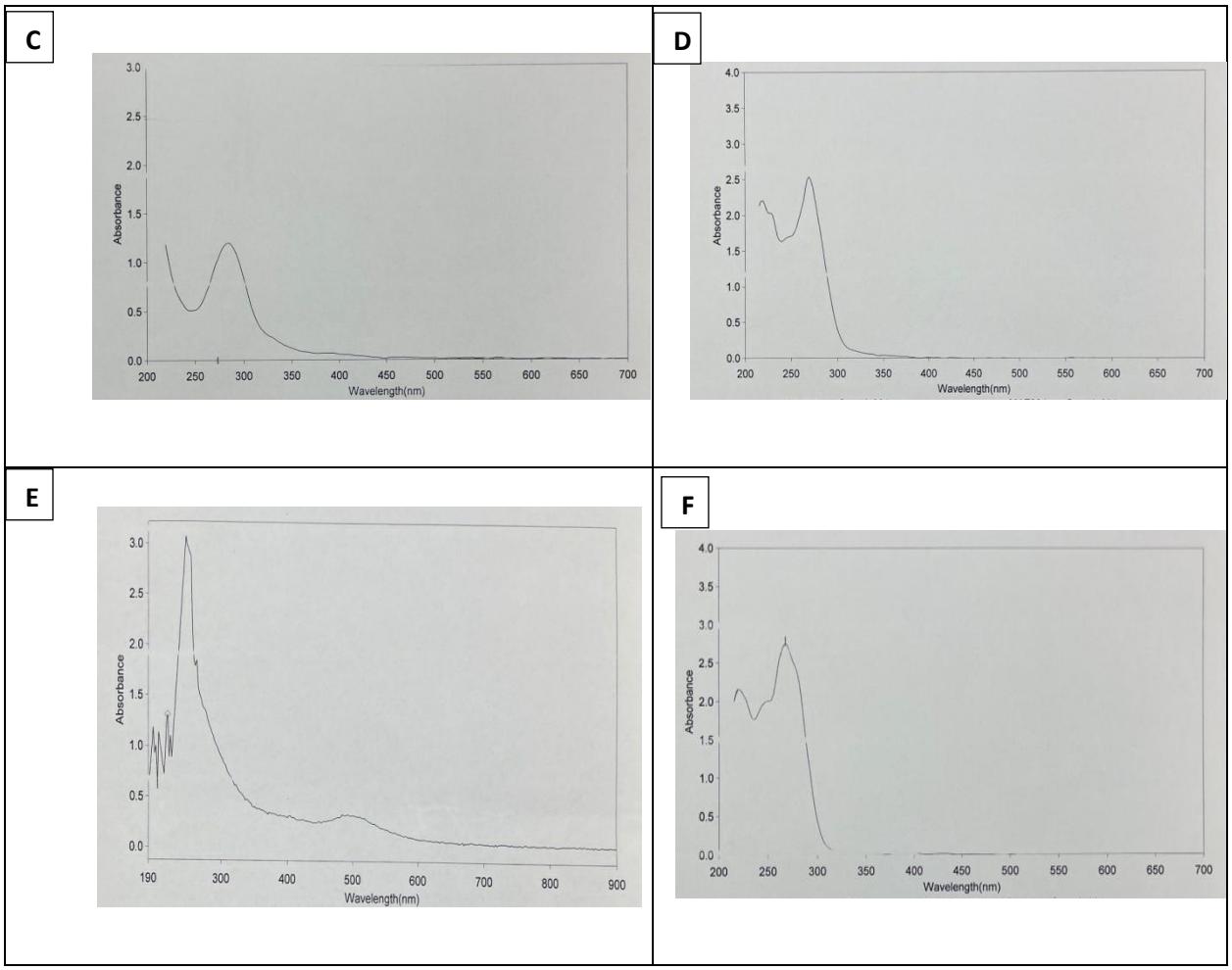


Fig. 3: UV scanning graphs showing absorbance peaks of the produced fungal pigments from different fungal isolates; A) medium control, B) Aspergillus terreus (At6), C) Alternaria alternata (Alt2), D) Aspergillus flavus (Af2), E) Penicillium purpurogenum (PP2), and F) Penicillium citrinum (Pc).

CONCLUSION

The current study revealed a higher diversity of rhizosphere fungi than rhizoplane, phyllosphere and phylloplane associated with the selected plants. As well as this study showed the potentiality of different fungal species for natural pigment production on liquid medium. Among the thirty fungal species evaluated, nineteen were able to synthesize coloured compounds. *Penicillium purpurogenum* exihibited the highest productivity of fungal pigments, producing a red colored pigment as extracellular secondary metabolite in the culture broth. Thus, this pigment could be easily harvested without breaking of the fungal biomass and expensive downstream processes. So, more research is required to identify the fungus and analyze the characteristics of the red colored pigment in order to apply in various industries. From the previous results and owing to the different benefits of fungal pigments. We recommend the use of this safe and natural pigment in different industries.

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