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Morphological characterization of plant growth promoting fungi (PGPF) isolated from maize rhizosphere in Meghalaya

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Abstract
There has been a resurgence of interest in the quest for Plant growth-promoting fungus (PGPF) for sustainable crop production in recent years. PGPF are heterogeneous collection of nonpathogenic fungi that are connected with plant and mediate improvements in plant development and health. In agriculture, the usage of PGPF is constantly expanding, and it offers an appealing alternative to chemical fertilizers and pesticides. The queen of cereals, maize is one of the most important cereal crop, and it is susceptible to a variety of fungal, viral and bacterial pathogens. Through extensive surveys conducted in different maize growing areas of Meghalaya, soil samples were collected from maize rhizosphere region from eight (8) districts *viz.*, Ri-bhoi, West Garo Hills, North Garo hills, West Khasi Hills, West Jaintia hills, South West Garo Hills, East Khasi Hills and East Jaintia Hills. A total of fourty (40) fungi were isolated and recovered from rhizospheric soil by serial dilution method, of which majority of fungal isolates belonged to the genus *Trichoderma* sp., followed by *Penicillium* sp., *Aspergillus* sp, *Phoma* sp., *Fusarium* sp., *Aspergillus* sp., *Chaetomium* sp., *Metarhium* sp. and *Acremonium* sp. Further, these fourty (40) isolates were identified based on morphological characters. Further research can be done to find the antagonistic capability of rhizospheric fungus against the major fungal diseases of maize.

Keywords: Maize, morphological characterization, plant growth promoting fungi (PGPF), rhizosphere

Introduction
Maize (*Zea mays* L.), the queen of cereals, is the world's third most important cereal crop and a significant source of food, feed and industrial products (Singh and Shahi, 2012) [19]. After rice and wheat, maize covers an area of 9.47 million hectares in India, with a total production of 28.72 million tonnes and a productivity of 3032 kg/hectare (Directorate of Economics & Statistics, DAC&FW, 2018) [1]. In maize, diseases are expected to cause loss of 13.2% of maize's economic product every year according to reports (Prasanna *et al*., 2009) [16]. Various diseases affect the crop in the hot humid foothill region of Himalayas and the plains which includes the states of Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, Meghalaya, Assam, Nagaland, Punjab, Haryana, Rajasthan, Madhya Pradesh, Karnataka, Delhi, Uttar Pradesh and Bihar (Payak and Sharma,1981) [15]. Maize cultivation has become one of the special farming system in Meghalaya, where most of the farmers grow during both kharif and Rabi seasons. Maize is the second most important food crop of Meghalaya next to rice occupying around 18,000 ha area (8% of total area) with an average yield of 2,150 kg/ha (Babu *et al*., 2019) [3].

Plant growth promoting fungi (PGPF) are a broad group of nonpathogenic fungi found in plants that mediate favourable changes in plant development and health. Majority of the fungi classified as PGPF belongs to the phylum Ascomycota (*Aspergillus, Aureobasidium, Chaetomium, Cladosporium, Colletotrichum, Exophiala, Penicillium, Trichoderma, Fusarium, Gliocladium, Phoma, Phomopsis, Purpureocillium,* and *Talaromyces*) and a few of them belonging to Basidiomycota (*Limonomyces, Rhodotorula, Rhizoctonia* and sterile fungi) and Zygomycota (*Mucor* and *Rhizopus*). Most of the PGPF is originated either from soil or roots of massive host. Plant diseases can be suppressed by PGPF in a variety of ways, which includes antibiotic production and pathogen competition for food and space. Plant growth enhancement by PGPF can also result from increased nutrient availability, alleviation of abiotic stressors and antagonism of phytopathogen (Wakelin *et al*., 2004; Hossain *et al*., 2017) [24, 11]. Chemical fertilizers and pesticides had a huge negative impact on the environment. These substances are

hazardous and have the potential to persist and accumulate in natural ecosystems. Replacing chemicals with biological approaches, which are deemed more environmentally beneficial in the long run, is one solution to this problem. The use of biocontrol plant growth promoting fungi (PGPF), which are capable of inhibiting or preventing the damage of phytopathogen, is one of the developing research areas for the control of phytopathogenic agents. Therefore, characterization, identification and adoption of new and native biopesticides will be helpful for the farmers and the whole community by avoiding use of chemicals.

Materials and Methods

Collection of soil sample

Soil samples from maize rhizosphere were collected from 27 locations and eight (8) districts of Meghalaya *viz*., Ri-Bhoi, East Khasi Hills, West Garo Hills, North Garo Hills, West Khasi Hills, South West Garo Hills, West Jaintia Hills and East Jaintia Hills. Soil samples were taken from the root zone of maize to a depth of 10-20 cm around rhizosphere of healthy maize plant. The method of sampling was done randomnly using stratified random sampling. Soil samples were packed in a clear plastic bag with zipper which was then labelled and brought to the laboratory of department of plant pathology, School of Crop Protection (SCP), CPGS-AS, CAU, Meghalaya and stored in a refrigerator at 4℃ until further processing which normally was within 24 hours of collection. The collected soil samples were sieved (2 mm mesh) to remove gravel and plant debris, and then, made air dried for 24 hours at room temperature.

Isolation and maintenance of fungal isolates

Fungal isolates were isolated from rhizosphere soil of healthy maize plants by serial dilution technique given by Rabeendran *et al*. (1998) with slight modifications. Ten gram of soil was suspended in 250 ml Erlenmeyer flasks containing 100ml sterilized distilled water and then, homogenized by using a rotary shaker at 250 rpm for 20-30 minutes. Each soil suspension was serially diluted to obtain dilution factor from 10^{-1} to 10^{-5}. From each dilutions, an aliquot of 0.1 ml of substrate suspension was taken with the help of a micropipette and dispensed into sterilized Petri plates (90 mm in diameter) containing Potato Dextrose Agar (PDA) medium, (Hi-media Ltd., Mumbai). Then, the Petri plates were incubated at 27±3℃ for one week. Morphologically distinct colony was isolated, purified and grown in pure culture on PDA.

The obtained pure cultures were maintained by regular sub-culturing every two months in PDA slants and stored at 4 °C in refrigerator for short term storage. For long term storage, fungal mycelial disc was kept in PDA slant with 20% glycerol and in mineral oil at -20°C.

Identification and Characterization

The fungal isolates were studied based on morphological (colour, growth and texture) (Bisset, 1992; Raper and Thom, 1949) [5, 18] and microscopic characteristics (mycelium, conidiophore, spore structure etc). under microscope (Leica ICC50, Germany). Cultural characteristics of fungal isolates were identified with the help of Royal Horticultural Society Colour Charts Edition V, London. Also those unidentified dominant species were sent to National Centre of Fungal Taxonomy (NCFT), New Delhi for identification.

Result

Isolation of fungal isolates from soil

Soil samples were collected from healthy maize rhizosphere from 27 different locations of 8 districts of Meghalaya (Table 1). Isolation of fungal isolates were by done by serial dilution method as described above. Altogether, fourty (40) fungal isolates were isolated and results are shown in Table 4.1. It was observed that out of fourty (40) isolates, the maximum number of fungal isolates were isolated from Ribhoi district, followed by East Khasi Hills, West Garo Hills, South West Garo Hills, West Jaintia Hills, West Khasi Hills, East Jaintia Hills and least number was observed in North Garo Hills.

Morphological characterization and identification of fungal isolates

Fungal isolates isolated from soil samples of different districts of Meghalaya were morphologically different. Fungal isolates showed variation in their morphological characters like colony colour, growth, texture, margin when grown in PDA media. Shape and colour of conidia and conidiophore also varied in different isolates when observed under microscope. Out of fourty (40) isolates, *Trichoderma* sp. (11 isolates) were identified in maximum as compared to other fungal isolates which was followed by *Penicillium* sp. (8 isolates), *Phoma* sp. (7 isolates), *Fusarium* sp. (5 isolates), *Aspergillus* sp. (3 isolates), *Chaetomium* sp. (2 isolates), *Metarhizium* sp. (2 isolates), and least number of *Acremonium* sp. (1 isolate), and *Pythium* sp. (1 isolate), The details of the morphological and microscopic characters of fungal isolates are given in Table 2 and Plate 1a and b, Plate 2a and b, Plate 3a and b, Plate 4a and b.

Table 1: Total fungal isolates collected and isolated from maize rhizosphere in different districts of Meghalaya

Sl. No.	District	Location	GPS coordinates
1	West Khasi Hills	Nongstoin	25° 31' 1.34" N 91° 15' 53.42" E
		Mairang	25° 33' 41.94" N 91° 38' 9.67" E
2	East Khasi Hills	Umshing	25.6076ºN 92.5805ºE
		Mattilang	25.5396ºN 91.8216ºE
		Myllem	25º30’20.1348’’N 91º48’45.234’’E
		Mawryngkneng	25º33’7.6356’’N 92º3’49.8816’’E
		Lyngkien	25.4598ºN 91.7244ºE
3	West Jaintia Hills	Ummulong	25º31’4.962’’N

			92º9’20.7612’’E
		Thadlaskein	25º30’8.9784’’N 92º10’22.1088’’E
		Mawkyndeng	25.5146ºN 92.3949ºE
4	East Jaintia Hills	Daistong	25.3398ºN 92.5805ºE
		Moolasngi	25.3761ºN 92.4456ºE
5	West Garo Hills	Damalgiri	25.7003° N 90.0494° E
		Chibinang	25.8669° N 90.0817° E
6	South West Garo Hills	Sulguri	25.5010° N 89.9492° E
		Chelipara	25º28’23.8944’’N 89º57’49.86’’E
		Dufrigaon	25° 28’ 9.8148‘’ N 89° 57’ 4.7664" E
		Tangabari	25.9949° N 90.7985° E
7	North Garo Hills	Mendipathar	25.9158° N 90.6441° E
8	Ribhoi	CPGS-AS	25.535410° N 91.277690° E
		Umsning	25° 44' 41.4924" N 91° 53' 12.5628" E
		Bhoirymbong	25° 42' 27.9288" N 92° 1' 20.658" E
		Mawpun	25º42’27.738’’N 91º56’56.202’’E
		Umeit	25º42’45.7128’’N 91º57’20.394’’E
		Umtrew	25° 43' 18.1272" N 91° 53' 24.5544" E
		Umjarasi	25.8794° N 91.8859° E
		Pyllun	25º42’45.826’’N 91º57’20.1716’’E
	Total	27	

Table 2: Morphological and cultural identification of fungal isolates on PDA medium

SL. NO.	Isolates	Cultural characteristics					Microscopic observation	
		Colour		Growth	Texture	Margin	Mycelium and conidiophore	Conidia
		Front	Reverse					
1	*Acremonium falciforme*	Creamy white	Dull white	S	Co	I	Hyphae is hyaline, having simple phialides	Conidia hyaline, cylindrical and single celled
2	*Aspergillus flavus*	Yellow green	Pale white	F	P	I	Mycelium is septate and hyaline. Rough and colourless conidiophore	Round shaped green coloured conidia, arranged in a long chain.
3	*Aspergillus niger*	Dark black	Pale white	F	P	I	-do-	Round shaped black coloured conidia, arranged in a long chain.
4	*Aspergillus* sp.	Black	Pale white	F	P	I	Mycelium septate, branched and hyaline. Erect and club shaped conidiophore	Round shaped black conidia
5	*Fusarium oxysporum*	Purple white	Pink	M	Co	C	Septate and branched mycelium Conidiophores are elongated and lightly branched	Microconidia oval to kidney shaped
6	*Fusarium pallidoroseum*	Light pink	Creamy yellow	M	Co	C	3-7 septation and short conidiophores	Curved macroconidia, ellipsoid microconidia
7	*Fusarium verticillioides*	pink	Light yellow	M	Co	C	Septate and hyaline mycelium. Conidiophores medium length, simple and lightly branched	Macroconidia are sparse (5 septation), very slightly sickle shaped. Microconidia abundant (0 to 1 septation)
8	*Fusarium solani*	White	Off white	M	Co	I	Phialides and chlamydospores are present	Macroconidia are slightly curved and hyaline. Microconidia are cylindrical, hyaline and aseptate.
9	*Fusarium* sp.	Creamy yellow	Creamy yellow	M	Co	C	Mycelium septate and branched. Phialides are present	Macroconidia with 3 septa and microconida single-celled
10	*Metarhizium anisopliae*	Olive Green	Light yellow with zones	S	S	C	Mycelium septate and branched. Conidiophores in candle- or palisade-like arrangement, Phialides elongate, cylindrical	Single-celled, intermediate and small sized ellipsoid conidia
11	*Metarhizium* sp.	Dark green	Creamy yellow	S	V	C	-do-	Hyaline, cylindrical conidia with round edges
12	*Penicillium* sp.	Olive green with concentric circles	Creamy	S	FV	I	Hyphae septate, branched and hyaline. Erect and unbranched conidiophore	One-celled and globose conidia
13	*Penicillium expansum*	Blue green	Creamy	M	V	C	Branched and septate conidiophores	Granulat to floccose conidia. Phialides are flask shaped

14	*Penicillium* sp.	Light Blue	Creamy with reddish pigments	M	FV	C	Hyphae septate, branched and hyaline. Erect and unbranched conidiophore	One-celled and globose conidia
15	*Penicillium* sp.	Blue green	Creamy	M	FV	C	Septate hyphae, branched conidiophores, swollen phialides	Oval conidia produced on smooth-walled conidiophores
16	*Penicillium chrysogenum*	Bluish green with yellow pigment	Creamy	M	P	C	Brush shaped conidiophores, septate hyphae	One-celled conidia
17	*Penicillium rubens*	Green yellow	Creamy	M	V	I	Septate hyphae and smooth conidiophore	Conidia is smooth walled, ellipsoid
18	*Penicillium citrinum*	Grey white with secretion of red gel like fluid	Creamy	S	FV	I	Hyphae septate, branched and hyaline. Erect, unbranched and septate conidiophore	One-celled hyaline and globose conidia
19	*Penicillium* sp.	Light blue	Creamy with reddish pigments	S	FV	I	-do-	-do-
20	*Pythium* sp.	White	White	F	CF	C	Coenocytic, hyaline hyphae	Thick walled oospores and lobed sporangia
21	*Chaetomium globosum*	Olive green	Creamy	M	P	I	Mycelium grows in conglomerate masses like ropes. Ostiole dark with unbranched radiating hairs	Flat lemon-shaped and olive brown ascospores within clavate ascomata
22	*Trichoderma harzianum*	White	White	F	P	R	Flask shaped phialides and arranged in divergent groups of 2-4	Globose to subglobose conidia
23	*Trichoderma viride*	Green yellow with concentric rings	Pale white	F	P	C	Slender phialides	Globose conidia
24	*Trichoderma hamatum*	Green white	Creamy	F	P	C	Septate branched with flask shaped phialides	Globose shape with smooth walled conidia.
25	*Trichoderma koningii*	Green white	Yellow	F	P	C	Elongated phialides	Oblong ellipsoidal conidia
26	*Trichoderma attroviride*	Blue green	Pale white	F	P	C	-do-	-do-
27	*Trichoderma harzianum*	Green	Pale white	F	P	C	Flask shaped phialides and arranged in divergent groups of 2-4	Globose to subglobose conidia
28	*Trichoderma* sp.	light green	Pale white	F	P	C	Mycelium hyaline with many branches in the edge	Hyaline and oval conidia
29	*Trichoderma ghanense*	Dark green	Orange	F	P	C	Septate branched with flask shaped phialides	Ellipsoidal, typically smooth with smooth walled conidia.
30	*Hypocrea nigricans*	Green	Pale white	F	P	C	Flask shaped phialides and arranged in divergent groups of 2-4	Globose to subglobose conidia
31	*Trichoderma* sp.	White	Yellow	F	P	C	Hyaline with many branches in the edge	Oval to elongate conidia
32	*Trichoderma lixii*	Dark green	Creamy	F	P	C	Septate branched with flask shaped phialides	Ellipsoidal, typically with smooth walled conidia.
33	*Trichoderma inhamatum*	Yellow white	Pale white	F	P	C	Flask shaped phialides	Globose, typically smooth with smooth walled conidia.
34	*Phoma herbarum*	Grey white	Grey	F	Co	I	Hyphae septate and hyaline. Pycnidia	Single celled hyaline, ovoid to ellipsoidal conidia. Conidia are bi-guttulate
35	*Phoma* sp.	Grey white	Grey	F	Co	C	Hyphae septate and hyaline. Chlamydospores are unicellular, dark brown and botryoid-alternarioid shape. Pycnidia grey, globose and ostiolate	Conidia ellipsoidal to cylindrical, smooth, hyaline and aseptate
36	*Phoma glomerata*	Pale pink with greyish centre	Pale pink	F	WC	C	Dark brown septate hyphae. Chlamydospores in branched or unbranched chains.	Conidia ellipsoidal, smooth, hyaline and single celled
37	*Phoma lingam*	White	White	F	Co	C	No chlamydospores, dark walled pycnidia	Oblong to elliptic or often irregular hyaline conidia
38	*Phoma sorghina*	White grey	Grey	F	Co	I	No chlamydospores, dark walled pycnidia, ostioles often with short beaks	Oblong to cylindrical with rounded ends hyaline conidia
39	*Phoma* sp.	White grey	Grey	F	Co	I	-do-	-do-
40	*Phoma* sp.	Pale pink	Red	F	Co	C	Mycelium hyaline and septate. Pycnidia dark brown and occur singly.	Conidia oblong to ovoid, hyaline and aseptate

Note: Sl= Slow; F=Fast; M= Medium; C=Circular; I= Irregular; R= Regular; Co=Cottony; P=Powdery; S=Smooth; V=Velvety; FV=Flat and Velvety; CF= Cottony and Fluffy; WC= Wooly colonies

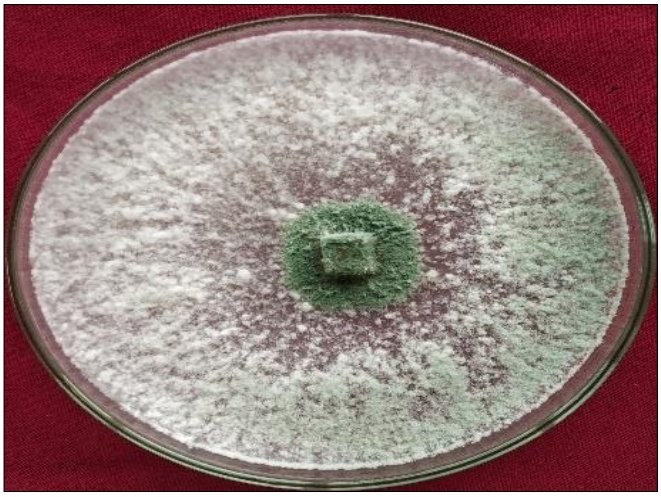

Plate 1a: Pure culture of *Trichoderma hamatum* on PDA

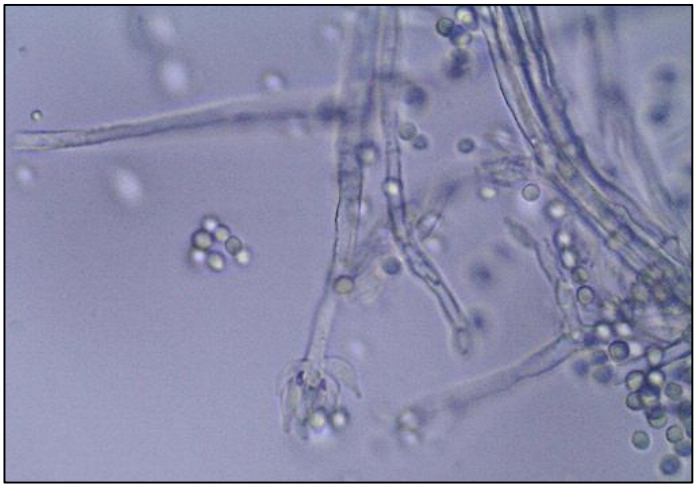

Plate 1b: Conidia and conidiophore of *T. hamatum* under 40X

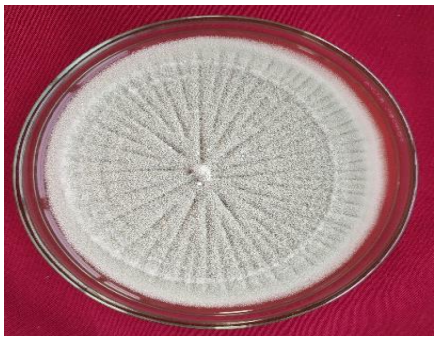

Plate 2a: Pure culture of *Penicillium expansum* on PDA

Plate 2b: Conidia and conidiophore of *P. expansum* under 40 X

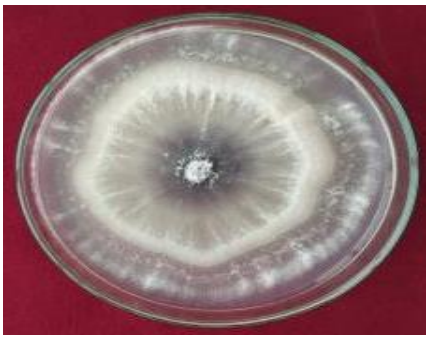

Plate 3a: Pure culture of *Fusarium oxysporum* on PDA

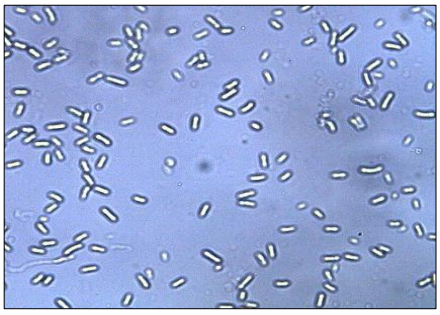

Plate 3b: Spores of *F. oxysporum* under 40 X

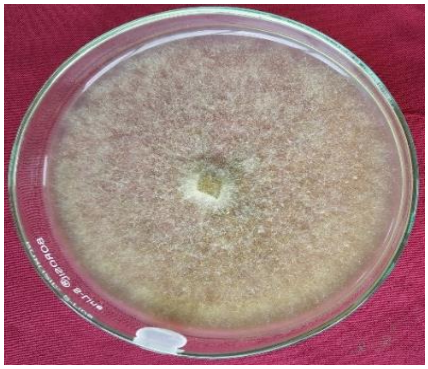

Plate 4a: Pure culture of *Chaetomium globosum* on PDA

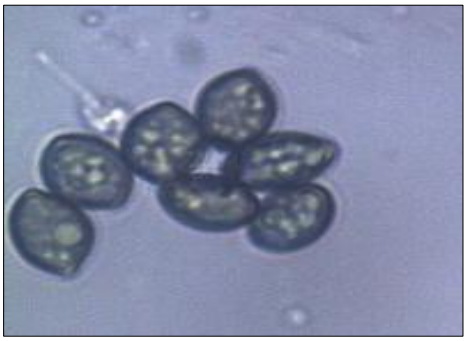

Plate 4b: Spores of *C. globosum* under 40 X

Discussion

There are many reports on the isolation of fungi from the rhizosphere of several crops. To get a taxonomic description of a taxon, morphological study and isolation techniques are important (Sun and Guo, 2012; Fernandes *et al*., 2015) [22, 9]. The methods for counting microorganisms by serial dilution (for isolating fungi) reveal that imperfect fungi predominate (Garret, 1976) [10]. Beneficial effect in number of rhizospheric fungi with respect to PGPF has been known since long ago (Hyakumachi, 1994) [12]. Most of the fungi which belong to PGPF are the genus *Aspergillus, Aureobasidium, Chaetomium, Cladosporium, Penicillium, Trichoderma, Fusarium, Gliocladium, Phoma* etc. Many fungi has been reported as PGPF along with the source of isolation. Some of them are *Pythium oligandrum* from soil (Benhamou *et al*., 2012) [4], *Penicillium chrysogenum* from soil and cereal crops (Jogaiah *et al*., 2013) [13], *Phoma multirostrata* from rhizospheric soil and cereal crops (Jogaiah *et al*., 2013) [13], *Trichoderma asperellum* from soil (Yedidia *et al*., 2001) [25], *T. atroviride* (Contreras-Cornejo *et al*. 2011) [8] and *T. hamatum* from soil (Shaw *et al*., 2016) [21], *T. harzianum* from rhizospheric soil and cereal roots (Hyakumachi, 1994 [12]; Brotman *et al*., 2013 [6]; Jogaiah *et al*., 2013 [13]; and Akhter *et al*., 2015 [2], *T. virens* from soil (Contreras-Cornejo *et al*., 2009) [7]. Studies in this experiment revealed that majority of the organisms obtained from maize rhizosphere were *Trichoderma, Penicillium, Pythium and Fusarium* after isolation by serial dilution method. Similar results revealed in a study were majority of organisms obtained from screening included fungi like *Penicillium, Trichoderma*, some species of *Fusarium* and *Aspergillus* in Kenya from bacterial wilt endemic areas (Kones *et al*., 2020) [14]. Also in another study, *Trichoderma, Aspergillus*, and *Fusarium* spp. populations were similar to those found by Thormann and Rice (2007) [23], which identified *Trichoderma* spp. as one of the most common fungus in the rhizosphere.

The fundamental attributes in myco-taxonomy are phenotypic features, which provide a basic figure of an unknown taxon. Cultural characteristics comprising growth rate, colony colour and colony appearance were regarded as taxonomically relevant characteristics for fungal isolates like *Trichoderma* (Samuels *et al*., 2002a) [20]. Studies in this experiment revealed that most of the *Trichoderma* isolates varied in colour (White, green, yellow white etc) and conidia shape but did not vary much in texture, margin and conidiophores. The same findings like pale or yellowish colour of reverse of colonies were observed in a study in *Trichoderma* isolates and were recorded (Samuels *et al*., 2002a) [20].

Conclusion

over the last decades, a large number of fungal and fungus-

like species have been found. As a result, there has been, as well as the creation of new tools and approaches in the field of fungal systematics. Morphology provides a particular dimension to the taxonomy of a species and nowadays these are supported with DNA sequence based phylogeny and its evolutionary relationships can be interpreted. The study reveals that the morphological characterization of fungal species *Trichoderma*, *Penicillium*, *Phoma* and Fusarium, as the most abundant fungi found in the rhizosphere of maize. Though morphological characteristics can be used to show the dissimilarity between rhizospheric microorganisms, additional DNA- based approaches are required to demonstrate their evolutionary relationships.

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