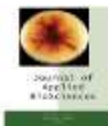

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Identification and phylogenetic relationship of fungi species associated with potato aphids in Bamenda, Northwest Region of Cameroon

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ABSTRACT

Objective: Potato (*Solanum tuberosum* L.) is one of the world's most important cultivated tuber crops in Cameroon. Potato aphids remain a major pest to potato plant thus greatly reducing its productivity. Inadequate information still exists on identification of fungi species associated with potato aphids. The aim of the study was therefore to identify fungi species associated with aphids of potato using molecular techniques to determine the entomopathogenic species which can be used to control potato aphids to increase potato productivity as well as increase food security.

Methodology and Results: One hundred samples of aphid's cadavers were collected monthly from the field, put in zip lock bags and preserved in coolers. These samples were then transported to the laboratory and cultured on potato dextrose agar. After a period of 7 days, they were sub-cultured to obtain pure cultures. The pure cultures were obtained and molecularly identified using the ribosomal ITS and TEF regions. Results from cultural identification revealed fungi belonging to three genera: *Fusarium, Aspergillus* and *Penicillium* with different species in these genera. Sequence data analysis from the ITS gene regions revealed 6 fungi species namely, *Fusarium oxysporum Aspergillus sydowii, Aspergillus niger, Curvularia affinis, Microascus murinus and Trichoderma erinanaceum.* Also, 6 species with the translation elongation factor (TEF) were identified namely, *Cladosporium cladosporoides, Fusarium oxysporum, Fusarium babinda, Trichoderma gamsii, Chaetomium cochiloides and Aspergillus niger.* Phylogenetic analysis produced a phylogram consisting of sequences of samples collected from the study area together with those from the GenBank.

Conclusions and application of results: Some of these fungi species have been reported to be entomopathogenic. Further research will screen entomopathogenic isolates that will serve as a biocontrol strategy against potato aphids which is an environmentally friendly method of pest control compared to synthetic pesticides.
Keywords: Potato, Aphids, Fungi, Identification, Cultural, Phylogeny.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important cultivated tuber crop (FAO, 2009). According to the FAO (2009), potato is grown in over 125 countries, consumed by over a billion people worldwide. In developing countries, millions of people depend on potato for survival (Njomo *et al*., 2019). The ease of cultivation of potatoes and its nutritional value (such as cholesterol free, rich in dietary fibres, antioxidants and high potassium content) are responsible for the expansion of potato production in developing countries. Production of potatoes in developing countries is still low, when compared to production in developed countries (FAO, 2009). In Cameroon, potato is an important crop and ranks among the major crops in tons produced after cassava, plantain, cocoyam, and maize, with majority production done by small holders mostly women, with most marketing done locally (Njomoh *et al*., 2019). Potato was introduced in Cameroon during the German colonial period between 1884 to 1914. Later in the 1940's, its cultivation became widespread by the British and Dutch government (Foncho, 1982). The West and Northwest Regions of Cameroon are the predominant areas of potato cultivation, with 80% (435,354 tons) of national production (Mengui *et al*., 2019). These two regions are located in the western highlands of the country, characterized by cool temperatures, and high rainfall of at least 800 mm per annum at altitudes ranging between 900 to 3000 meters above sea level. About 17% is exported to neighbouring countries like Gabon, Central African Republic, Congo, and Equatorial Guinea (Mengui *et al*., 2019). Planting is usually done in March and harvesting in June. Second planting occurs in November depending on the water stored in the soil (Mengui *et al*., 2019). Potato production is still constrained by poor farming practices, pests, inefficient use of available technologies, poor soil fertility, high cost of inputs like fertilizers, low quality seeds and fungicides, lack of access to credit, and a lot more despite government subsidization (Mengui *et al*., 2019). The common insect pests of potatoes are the cutworms, fruit worms, leaf miners, white flies and aphids experienced at various growth stages (Konje *et al*., 2019), Potato aphids known as green peach aphids are usually prominent at the maturity stage.

Aphids (Aphididae) are one of the most destructive insect pests in farming (Francis *et al*, 2022). Some important infesting aphids of potato are the green peach aphids *(Myzus persicae)* and potato aphids *(Marcrosiphum euphorbiae)* which can be differentiated by their tubercles between the lower surfaces of their antenna. Those of *M. euphorbiae* slope externally while those of *M. persicae* rather converge (Yi and Gray, 2020; Francis *et al*., 2023). These species are highly polyphagous and are widely distributed making them able to attack many plant families (Machado-Assefh *et al*., 2023). They mostly have two stages in their life cycle, which is the nymph and adult but, in some stages, they experience three developmental stages which are the egg, nymph and adult (Yi and Gray, 2020). A female adult can give rise to about 20 to 50 nymphs which mature within a period of 4 to 21 days in summer season. Some fungi have been noted to be useful pathogens of aphids (Francis *et al*, 2022). They usually form an endophytic association with the aphids which is said to be

pathogenic to the aphids. These categories of fungi are called entomopathogenic fungi (EPF) and so causing death to the host insect (Scorcetti, *et al*., 2007). Majority of the pathogenic fungi associated with aphids belong to the phylum Ascomycota and class Entomophthoromycota (Humber, 2012). Conventional fungal morphological identification relies on the examination of both microscopic and macroscopic features. However, morphological plasticity and genetic variability in the parameters employed to identify species usually cause a restriction to this identification, despite its importance (Paz *et al*., 2011). The advancement of molecular techniques in recent years has made it possible to develop quick, precise, and widely applicable methods for the detection and identification of various fungi. These methods are primarily based on the polymerase chain reaction (PCR) analysis of DNA. The internal transcribed spacers (ITS) and ribosomal DNA genes (rDNA) for example, make it possible to examine the evolutionary connections between various entomopathogenic fungal taxa. As such, it's critical to compare the outcomes of morphological and molecular analysis to increase accuracy in the identification of fungal isolates and assess their genetic variability (Hibbet *et al*., 2011). Some endophytic fungi such as *Metarrhizum* have been identified molecularly by amplification and sequencing of factor 1-alpha (EF-1α) and β-tubulin (Clifton *et al*., 2018), *Cladosporium species* (Bensaci *et al*., 2022), *Cordiceps* and *Beauveria bassiana* using the internal transcribed spacer (Tian *et al*., 2010). Other researchers focus only on morphological means by looking at the growth features and characteristics of the colonies on culture plates, making comparisons with those in literature and using identification keys. Some group of fungi isolated from potato aphids such as *Beauveria bassiani* and *Metarrhizum anisoplae* have been identified by this means (Ullah *et al*., 2022). These groups of fungi have been reported in forming endophytic pathogenic association with most insect pests, including aphids of which potato aphids have not been reported to be an exemption (Vu *et al*., 2007, Ullah *et al*., 2022, Bensaci *et al*., 2022, Nouh, 2019). In Cameroon, identification of some of these species have been done only by morphological means (Membang *et al*., 2021). It is therefore imperative that these fungi are properly identified to aid in molecularly taxonomy of fungi species.

MATERIALS AND METHODS

Study area: The study was carried out in Bamenda, Mezam division located in the North-West Region of Cameroon, (Figure 1) with a combined surface area of 391 km2, it is composed of three subdivisions: Bamenda I, Bamenda II, and Bamenda III and located between latitude 5.5734N and longitude 10.845E (Acho, 1998). Its terrain is composed of vast valleys and plateaus spaced apart, with Guinea Savannah-type vegetation and mild temperatures. A high scarp-oriented NE-SW divides the two topographic units (Neba, 1999). The upper plateau, which is mostly Bamenda I and makes roughly 10% of the city's total size, is situated above the cliff. Here, altitudes range from 1472 metres to 1573 metres (Parfait *et al*., 2023). There are two distinct seasons in this humid tropical highland climate: wet and dry. This place has extremely cold temperatures, which are greatest from January to March. Minimum and maximum temperatures range from 14.10ºC to 17.80 ºC . Humidity ranges from 39 to 90%, and rainfall occurs at a rate of 0.1 to 14.1 inches per hour (Parfait *et al*., 2023). According to Tita *et al.* (2012), the rainy season is often longer, lasting eight months from mid-March to mid-October, with a brief dry season of four months from mid-October to mid-March. It has a mean yearly temperature of 19.93 degrees Celsius.

The annual average precipitation ranges from 1700 to 2824 mm and exhibits fluctuations (Ayonghe, 2001). Along the many watercourses in the watershed, the town boasts a densely populated area and a strong hydrographical network (Parfait *et al*., 2023). Two study regions were used for this work: Santa and small Babanki. Santa Sub-Division is one of the seven administrative units of Mezam Division in the Northwest Region of Cameroon (CDP, 2011). It is found along longitude 9º 58' and 10º 18' East of the Greenwich Meridian and between latitude 5º 42' and 5º53' North of the Equator (Konje *et al*., 2019). Santa is situated 20 km to the south of Bamenda, the Northwest Regional capital and about 60 km North of Bafoussam, the regional capital of the West Region (CDP, 2011). Santa is the main gateway into the Northwest Region from the rest of the country. In 2008, the estimated population was 99851 and 90% is engaged in farming and agriculture (Fogwe, 2014). Small Babanki is a village in the Northwest Region, Mezam Division and Tubah Subdivision. It is also known as Kedjom Ketinguh which means "people who live under the rock". It is located 20km East of Bamenda, the regional capital. It is 1100 to 1800m above sea level. It has an annual rainfall of 2450mm and an annual temperature of 18 to 21 ºC (Ndikintum, 2009).

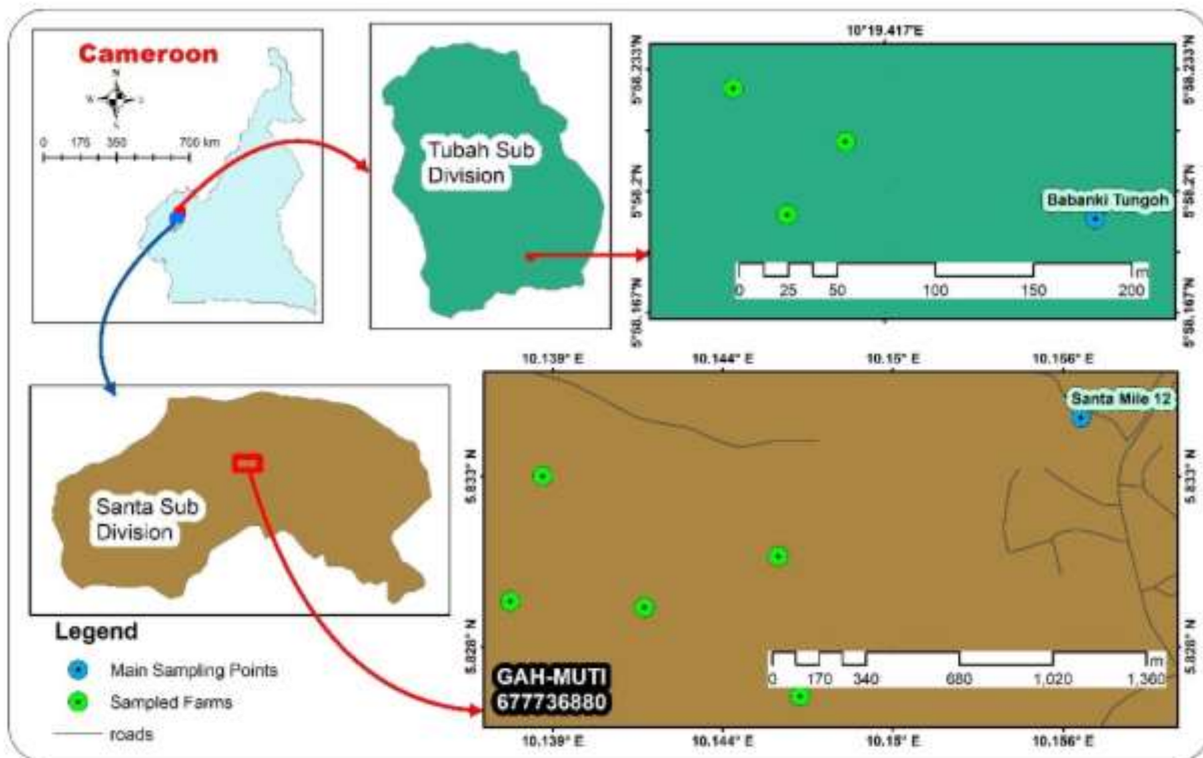

Figure 1: Map of the study area

Aphid collection: Field surveys were conducted in two villages, (Santa and Small Babanki) in November 2021 in Bamenda, Northwest Region of Cameroon. A total of 100 samples of insect cadavers suspected to have fungal infection were collected from the field (50 samples from each village site) with the help of an entomological forceps, put in labelled sample cups. These samples were put in coolers and transported to the Life Science Laboratory of the University of Buea, Southwest Region of Cameroon for isolation and identification of associated fungi.

Media preparation: The working environment was disinfected with 70% alcohol to reduce chances of contamination. Potato dextrose agar (PDA) medium was prepared following the protocol of Beever and Bollard, (1970)**.** The mixture was then homogenized, corked tightly with cotton, sealed with aluminium foil paper and autoclaved for an hour at 121°C and pressure of about 20 Pa. The medium was allowed to cool to about 40°C. An electronic wind balance was used to measure 0.1g of gentamycin while a micropipette was used to measure 500 mL of penicillin. These antibiotics were added to the PDA medium to prevent the growth of other microbes such as bacteria. The medium was gently stirred by swirling the flask and then poured into sterile petri dishes over a glowing flame from a gas bottle to prevent contamination from the environment**.** The medium was allowed to solidify before inoculation of the insect cadavers. Isolation of fungi: The insect cadavers were put in small nets, surface sterilized by immersing in 2.5 % sodium hypochlorite solution for 1 minute, followed by rinsing with 70 % alcohol for 1 minute. They were finally rinsed 3 times with sterile distilled water, then plated upon the solidified PDA medium in labelled Petri dishes (Mekwe *et al*., 2018). The Plates were sealed with parafilm and incubated at room temperature (25 °C) in the dark for 7 days. After 7 days, fungi that grew out of the inoculants were sub-cultured on fresh PDA plates at room temperature. The distinct fungi colonies from primary cultures were cut out using a sterile scalpel, and then transferred to the fresh PDA plates to obtain pure cultures. Inoculated plates were sealed with parafilm and incubated at room temperature (25 °C), in the dark for 7 days and pure cultures were obtained (Leslie and Summerrel, 2006). Morphological and cultural characterization: Macroscopic features such as colony diameter, colony colour, texture, margin, form, elevation and aerial hyphae were recorded by observing the growth forms on PDA physically. The mycelium was observed under a light microscope and crosschecked with those in literature (Leslie and Summerelle, 2006).

DNA extraction: DNA extraction was done following the Sorbitol-CTAB protocol according to Inglis *et al.* (2018). The oven was preheated at 65 °C. Before pipetting/dispensing, lysis buffer was warmed at 65 °C for at least 15 minutes. Fifty to 100mg of cultures that were stored in sterile distilled water was used. This was followed by the addition a bead mixture (3 spoons of 0.25-0.5mm), 1 spoon of 1-0.25mm and 2 glass beads 2.5mm) into each sample tube. The beater was beaded at 4m/s for 30sec x 2. An excess or 500 µl of sorbitol wash buffer (80 µl mecaptolethanol was added to every 40mL sorbitol wash before usage) was added to fill sample tubes containing macerated plant material to approximately ¾ capacity (0.9 – 1.5 ml, depending on tubes used). The tubes were caped and shook manually or using vortex. This stage was checked again to make sure that the powdered material formed a suspension, and the tubes were shaken again if need be. Centrifugation was done at 8500 rpm for 5 minutes at RT. The supernatant from samples was decanted or aspirated and discarded. Pre-warmed (at least 15mins) extraction buffer was added to the sample tubes (approximately ½ the sample tube capacity or 500 to 800 µl). To every 40mL CTAB buffer, 80 µl mecaptolethanol was added before usage. 5 µl proteinase K to was added to each sample tube. The macerated samples were resuspended by vortexing for 5 seconds. Samples were then incubated at 65°C for 60 minutes, with mixing by inversion every 10 minutes. The samples were removed from heating block and allowed to cool at RT for 5 minutes. They were centrifuged at 9000 rpm for 5 minutes at RT and 600 µl supernatant were removed and transfer into new 1.5mL tubes. CIA were added to sample tubes (600 µl or fill to approximately 4/5 tube capacity) and shook vigorously for 10

seconds. Centrifugation of the samples was done at 13000 rpm for 10 minutes at RT. The upper aqueous phase 500 µl was removed with much care by pipetting and transferred to a new 1.5mL tube, disturbance of the debris between phases was carefully avoided. A volume equivalent to 10% 3 M sodium acetate pH 5.2 (50 µl) and 70% of ice-cold isopropanol (stored at -20 °C) (350 µl) was added to each tube for the recovered supernatant to precipitate nucleic acids. This was mixed by inverting 10 times and kept at -20 °C overnight. Centrifuging was done at 13000 rpm for 10 minutes at RT. The supernatants were carefully decanted off and tubes drained by resting inverted on paper towels. The pellets were washed by the adding 1000 µl of 70 % ethanol. Centrifuge at 13,000 rpm for 10 minutes. The supernatants were carefully removed by aspiration to avoid loss of the nucleic acid pellet. The open tubes were dried at RT for approximately 1 hour. Pellets were suspended gently in 30 µl TE containing 0.1 mg ml^{-1} DNase-free RNase A and incubated tubes at 37 °C for 30 minutes. DNA purity was checked in a Nanodrop and stored at -20 °C for future use.

PCR amplification: The complete internal transcribed spacer (ITS) region, including ITS-1, ITS-2 and the 5.8S small subunit gene was amplified for all isolates using primers ITS1F and ITS4 (Hoggard *et al.,* 2018). Amplifications of the partial translation elongation factor 1-α (TEF1-α) gene region was obtained using primer pairs EF595F/EF1160R (Kauserud and Schumacher, 2001). The reaction was performed using GoTaq® (G2 Hot Start Colourless Master Mix Promega) and a primer combination of ITS1F (TCCGTAGGTGAACCTGCGG) and ITS4 (TCC TCC GCT TAT TGA TAT GC) with a total volume of 12 µl per reaction (Quecine *et al.,* 2014). The program was performed on the 'Primus 96' thermal cycler (MWG-Biotech, Ebersberg, Germany) and comprised an initial heat activation step at 95°C for 3 min, followed by 30 cycles of denaturation at 94°C (27 s), annealing at 57 °C (1 min), extension at 72 °C (1 min 30 s), and a final extension step at 72 °C (7 s). For TEF region, the PCR conditions used with primers EF595F (CGTGACTTCATCAAGAACATG) and EF1160R (CCGATCTTGTAGACGTCCTG) was as follows: an initial denaturation step at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s, 30 s of annealing at 55°C and 60 s of extension at 72°C. The reactions were completed with a final elongation step at 72°C for 7 min. PCR products were analysed using an ethidium bromide stained 1.5% w/v agarose gel (Inglis *et al*., 2018). QIAGEN® purification kit was used for the purification of the PCR products as instructed by the manufacturer. The purified PCR products were sent to for Sanger sequencing. The sequences obtained were edited using chromas software and a BLAST (Basic Local Alignment Search Tool) search was conducted in NCBI (National Center for Biotechnology Information) to obtain identical fungi with similar published sequences (Bich *et al*., 2021).

Phylogenetic Relationship: Alignment of the sequences downloaded from the GenBank was done using Mafft on the TrEase webserver (http://thineslab.senckenberg.de/trease/) implemented by the G-INS-i algorithm. The alignments were subjected to phylogenetic analysis using MEGA (Molecular Evolutionary Genetics Analysis) version 7.0 (Tamura *et el*., 2011). Phylogenetic inference was done using the Tamura-Nei substitution model using minimal evolution at 1000 bootstrap replicates and all other settings set as default.

RESULTS

Cultural Identification: Cultural identification revealed three different groups of fungi, identified as *Fusarium*, *Aspergillus* and *Penicillium* species (Figure 2).

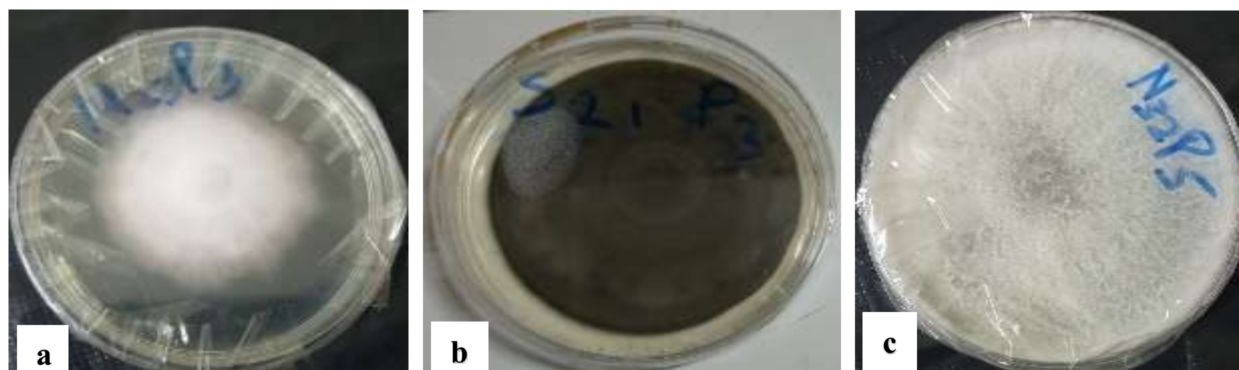

Figure 2: Three identified genera based on cultural features a: *Fusarium* b: *Aspergillus* c: *Penicillium*.

Aspergillus cultures had a colony dimeter that ranged from 21 to 80 cm, *Penicillium*, 78cm, *Fusarium*, 47 to 80 cm. Colony form of all 3 fungi were diverse from flat, raised to umbonate. The form of the colony varied from circular to irregular while the surface colony colour portrayed white, pink, green, creamy, ash, grey, black and fluffy white for all groups. The reverse colony colours were dark to yellowish and creamy. Table 1 shows the three groups of fungi identified using cultural characteristics.

Table 1: Fungi species identified morphologically using cultural characteristics.

Code	Suspected fungus	Colony diameter	Colony form	Colony margin	Surface colony colour	Reverse colony colour
G1	Unidentified	69	Flat	Circular	Ash	Dark
G2	*Fusarium*	80	Raised	Circular	White	Creamy
G3	*Fusarium*	76	Raised	Irregular	Pink	Violet
G4	Unidentified	66	Raised	Irregular	Fluffy-white	Creamy
G5	*Fusarium*	61	Raised	Irregular	Creamy	Yellowish
G6	*Penicillium*	78	Raised	Irregular	Green	Dark
G7	*Fusarium*	47	Umbonate	Irregular	Creamy	Yellowish
G8	*Fusarium*	53	Raised	Irregular	White	Violet
G9	*Fusarium*	47	Umbonate	Irregular	Peach	Orange
G10	*Fusarium*	54	Raised	Irregular	Peach	Orange
G11	Unidentified	72	Flat	Circular	Fluffy-white	Creamy
G12	Unidentified	76	Raised	Irregular	White	Cream
G13	*Aspergillus niger*	61	Flat	Circular	Black	Creamy
G14	Unidentified	71	Flat	Irregular	Black	Creamy
G15	Unidentified	76	Raised	Irregular	White	Creamy
G16	*Aspergillus*	72	Flat	Circular	Ash	Dark
G17	Unidentified	31	Flat	Irregular	Creamy/dark	Dark
G18	Unidentified	33	Flat	Circular	Grey/dark	Dark

G19	Unidentified	36	Umbonate	Circular	Ash	Dark
G20	Unidentified	44	Flat	Circular	White	Ash
G21	Unidentified	76	Flat	Irregular	Fluffy-White	White
G22	*Aspergillus niger*	39	Flat	Circular	Black	Creamy
G23	*Aspergillus fumigatus*	72	Raised	Circular	Ash	Dark
G24	*Aspergillus fumigatus*	44	Umbonate	Circular	Grey	Black
G25	*Aspergillus fumigatus*	65	Raised	Circular	Grey	Black
G26	*Aspergillus flavus*	50	Flat	Circular	Grey	Black
G27	*Aspergillus flavus*	38	Raised	Circular	Fluffy-white	Creamy
G28	*Aspergillus spp.*	46	Umbonate	Circular	Fluffy-white	Creamy
G29	*Aspergillus spp.*	60	Umbonate	Circular	Fluffy-white	Creamy
G30	*Aspergillus spp.*	81	Raised	Circular	Fluffy-white	Creamy
G31	Unidentified	16	Raised	Irregular	White	Creamy
G32	Unidentified	17	Raised	Irregular		Creamy
G33	*Aspergillus spp.*	37	Raised	Circular	Ash	Dark
G34	*Aspergillus spp.*	32	Raised	Circular	Ash	Creamy
G35	*Aspergillus niger*	62	Raised	Circular	Ash	Creamy
G36	*Aspergillus fumigatus*	23	Raised	Circular	Ash	Dark
G37	*Aspergillus flavus*	21	Flat	Irregular	Green	Creamy
G38	*Aspergillus fumigatus*	52	Raised	Circular	Green	Dark
G39	Unidentified	48	Raised	Circular	White	Creamy
G40	*Aspergillus spp*	59	Raised	Circular	Ash	Creamy
G41	*Aspergillus flavus*	31	Umbonate	Circular	Dark	Dark
G42	*Aspergillus fumigatus*	78	Raised	Circular	Dark green	Black
G43	*Aspergillus*	80	Flat	Circular	Black	Black
G44	*Aspergillus flavus*	49	Raised	Circular	Green	Creamy

Molecular Identification: BLAST search result of ITS sequences provided reference sequences from the GenBank with a maximum identity of 100%. The least maximum identity was 96.78%. The query coverage ranged from 79% to 100% (Table 2).

Table 2: BLAST search results for the ITS gene region

CODE	Scientific Name	Max Score	Total Score	Query Cover	E Value	Per. Ident	Acc. Length	Accession	Author
G1	*Fusarium oxysporum*	1007	1075	91%	0.0	99.82%	732	ON927009.1	Schltdl, 1824
G4	*Fusarium oxysporum*	1005	1005	98%	0.0	99.64%	865	MG574894.1	Schltdl, 1824
G5	*Fusarium babinda*	937	937	90%	0.0	100.00%	507	MT478951.1	Summerell *et al.*, 1995
G9	*Fusarium babinda*	937	937	90%	0.0	100.00%	507	MT478951.1	Summerell *et al.*, 1995
G10	*Fusarium tricinctum*	928	928	91%	0.0	99.41%	540	KR011974.1	Corda, 1838
G12	*Fusarium babinda*	937	937	79%	0.0	100.00%	507	MT478951.1	Summerell *et al.*, 1995
G13	*Chaetomium cochliodes*	1029	1029	97%	0.0	99.65%	575	OW982621.1	Palliser, 1910
G15	*Trichoderma gamsii*	1114	1114	100%	0.0	99.67%	857	KM491887.1	Samuels & Druzhinina, 2006
G19	*Fusarium oxysporum*	994	994	100%	0.0	99.45%	1419	LT970803.1	Schltdl, 1824
G21	*Aspergillus sydowii*	1048	1048	100%	0.0	99.65%	971	LN898726.1	Thom and church, 1926
G22	*Aspergillus niger*	1109	1109	100%	0.0	99.83%	76751	AM270051.1	Tieghem, 1867
G23	*Cladosporium xanthochromaticum*	1024	1024	98%	0.0	100.00%	1219	KY781767.1	Sandoval-Denis *et al.*, 2016
G24	*Cladosporium aciculare*	983	983	97%	0.0	99.44%	547	MZ568163.1	Bensch *et al.*, 2015
G26	*Cladosporium anthropophilum*	1024	1024	100%	0.0	100.00%	840	MF472932.1	Sandoval-Denis *et al.*, 2016
G28	*Periconia cookei*	826	826	86%	0.0	96.78%	497	MG333490.1	Mason and Ellis, 1953
G29	*Cladosporium xanthochromaticum*	1005	1005	98%	0.0	100.00%	593	MK732115.1	Sandoval-Denis *et al.*, 2016
G30	*Cladosporium colombiae*	1020	1020	97%	0.0	100.00%	553	ON920710.1	Schub and Crous, 2009

G33	*Curvularia affinis*	1053	1053	96%	0.0	100.00%	714	HG778981.1	Boedijn, 1933
G34	*Cladosporium cladosporioides*	1005	1091	99%	0.0	99.28%	1651	KJ596320.1	Fresen, 1850
G36	*Microascus murinus*	979	979	89%	0.0	100.00%	530	MG457819.1	Sandoval-Denis *et al.*, 2015
G37	*Penicillium chrysogenum*	1077	1077	99%	0.0	100.00%	608	MH127463.1	Thom, 1910

For the TEF gene region, a BLAST search results provided reference sequences form the GenBank with a maximum identity of 100%. The least maximum identity was 92.13%. The query coverage ranged from 69% to 100% (Table 3).

Table 3: BLAST search results for the TEF gene region

CODE	Scientific Name	Max Score	Total Score	Query Cover	E Value	Per. Ident	Acc. Length	Accession	Author
G5	*Fusarium babinda*	737	737	69%	0.0	99.51%	849	MZ954878.1	Held *et al.* (2021)
G6	*Neocucurbitaria rhamni*	475	475	41%	8e-129	92.94%	1207	MF795864.1	Jaklitsch *et al.* (2018)
G9	*Fusarium babinda*	793	793	79%	0.0	99.32%	908	MT502634.1	Summerell *et al.* (1995)
G10	*Fusarium babinda*	737	737	90%	0.0	99.51%	849	MZ954878.1	Summerell *et al.* (1995)
G12	*Fusarium babinda*	789	789	99%	0.0	98.87%	908	MT502634.1	Summerell *et al.* (1995)
G13	*Chaetomium cochliodes*	795	795	100%	0.0	99.32%	926	KF001720.1	Palliser, (1910)
G15	*Trichoderma gamsii*	758	758	93%	0.0	99.05%	960	FJ436186.1	Samuels and Druzhinina, 2006
G19	*Fusarium oxysporum*	749	749	95%	0.0	98.59%	539	MN190165.1	Schltdl, 1824
G23	*Cladosporium cladosporioides*	678	678	100%	0.0	94.74%	1075	OL697235.1	Fresen., 1850
G24	*Cladosporium sphaerospermum*	732	732	97%	0.0	97.45%	629	MT881852.1	Penzig, 1882
G26	*Cladosporium cucumerinum*	745	745	98%	0.0	97.91%	914	MW735657.1	Ellis and Arthur, 1889

G29	*Waydora typica*	562	562	99%	2e-155	90.26%	431	MN072433.1	Rodway, 1918
G30	*Cladosporium cladosporioides*	767	767	98%	0.0	99.07%	435	MK752020.1	Fresen., 1850
G32	*Aspergillus fischeri*	752	752	99%	0.0	97.72%	1383	XM_001265027.1	*Schoch, et al. (2020)*
G34	*Aspergillus welwitschiae*	717	717	99%	0.0	99.74%	1701	XM_026766727.1	*Schoch, et al. (2020)*
G36	*Cryptendoxyla consimilis*	606	606	99%	8e-169	92.13%	970	LT634054.1	Koukol, 2016
G37	*Cryptendoxyla consimilis*	606	606	99%	8e-169	92.13%	970	LT634054.1	Koukol, 2016
G40	*Aspergillus saccharolyticus*	619	619	99%	1e-172	92.26%	1479	XM_025575893.1	*Schoch et al. (2020)*
G42	*Cladosporium lebrasiae*	752	1211	96%	0.0	98.14%	598	LC425559.1	Vasseur and Crous, (2016)
G43	*Periconia delonicis*	734	734	100%	0.0	96.82%	920	MK360071.1	Jayasiri et al. (2019)
G44	*Penicillium chrysogenum*	623	623	96%	8e-174	93.18%	990	KP009000.1	*Schoch, et al. (2020)*

Phylogenetic relationship: Phylogenetic tree of the ITS sequences revealed six distinct fungal species: *Fusarium oxysporum Aspergillus sydowii, Aspergillus niger, Curvularia affinis,* Microascus murinus and *Trichoderma erinanaceum* with very high bootstrap support values of 93%, 100%,100%, 100%, 100% and 100% respectively. This gene region could not provide enough resolution of all our sequences to species level. Some sequences (G13, G24, G26, G30, G23, G19 and G27) could not be assigned to a specific species due to polytomy (the presence of many species in one single clade) observed in this clade. Some other sequences (G5, G9, G10, G12) could not be assigned to a specific genus but formed a clade with *Fusarium oxysporum* with a support value of 66%. One sister group was shown by Aspergillus sydowii, and *Aspergillus niger* with a support value of 97%. Figure 3 shows the minimum evolution tree for ITS sequences constructed with the Tamura-Nei model with 1000 bootstrap replicates.

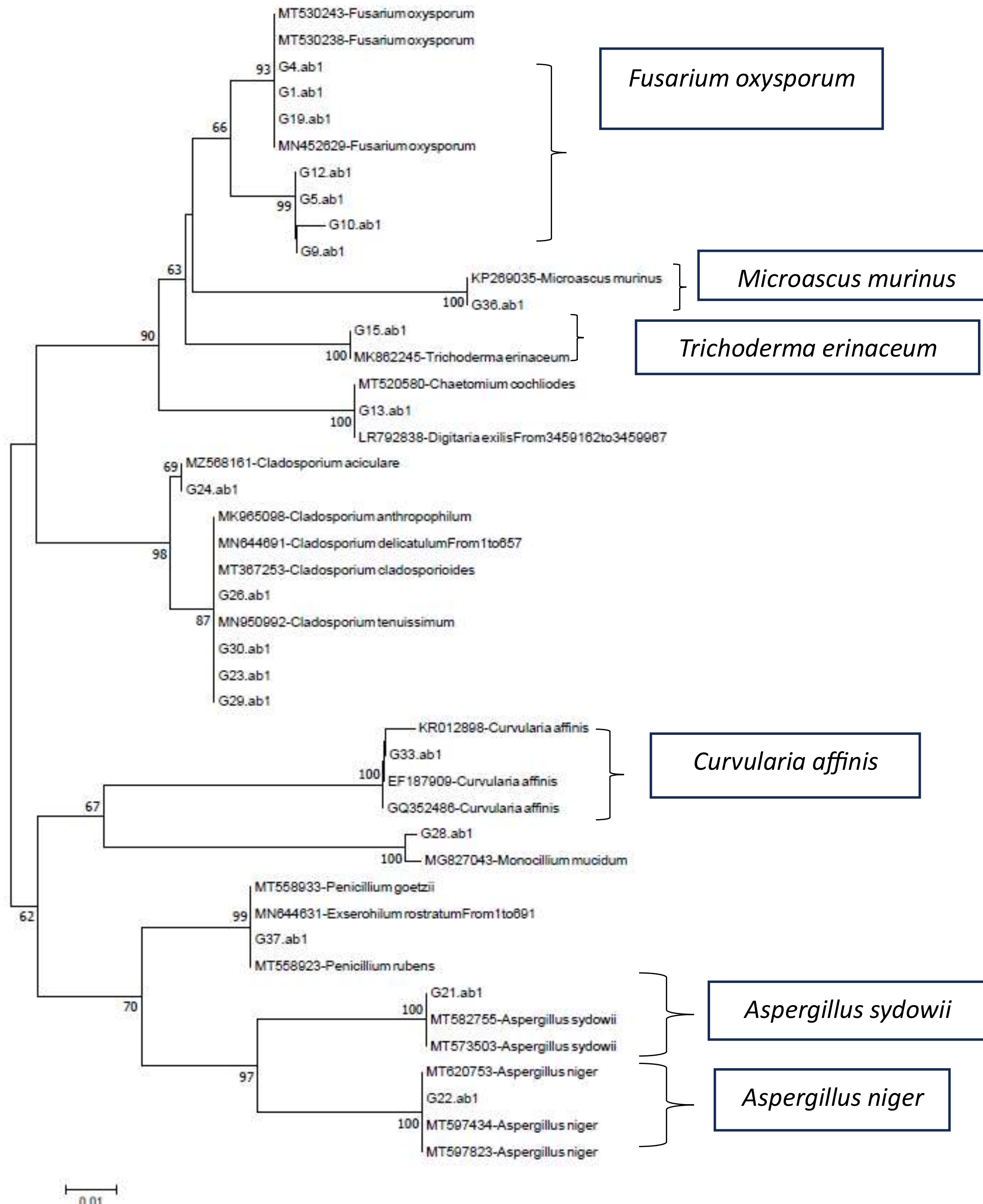

Figure 3: Minimum evolution tree constructed using the Tamura Nei model for the ITS gene region with reference sequences from the BLAST software. Number on branches denotes bootstrap values from minimum evolution analyses.

Phylogenetic tree of the TEF sequences showed 6 distinct species namely, *Cladosporium cladosporoides, Fusarium oxysporum, Fusarium babinda, Trichoderma gamsii, Chaetomium cochiloides and Aspergillus niger* with support values of 88%, 77%, 99%, 100%, 100% and 99% respectively. *Fusarium oxysporum* formed a sister group with *Fusarium babinda* with a 100% support value. This gene region was still not sufficient to identify all the sequences. Figure 4 shows the minimum evolution tree for TEF sequences using the Tamura-Nei model with 1000 bootstrap replicates.

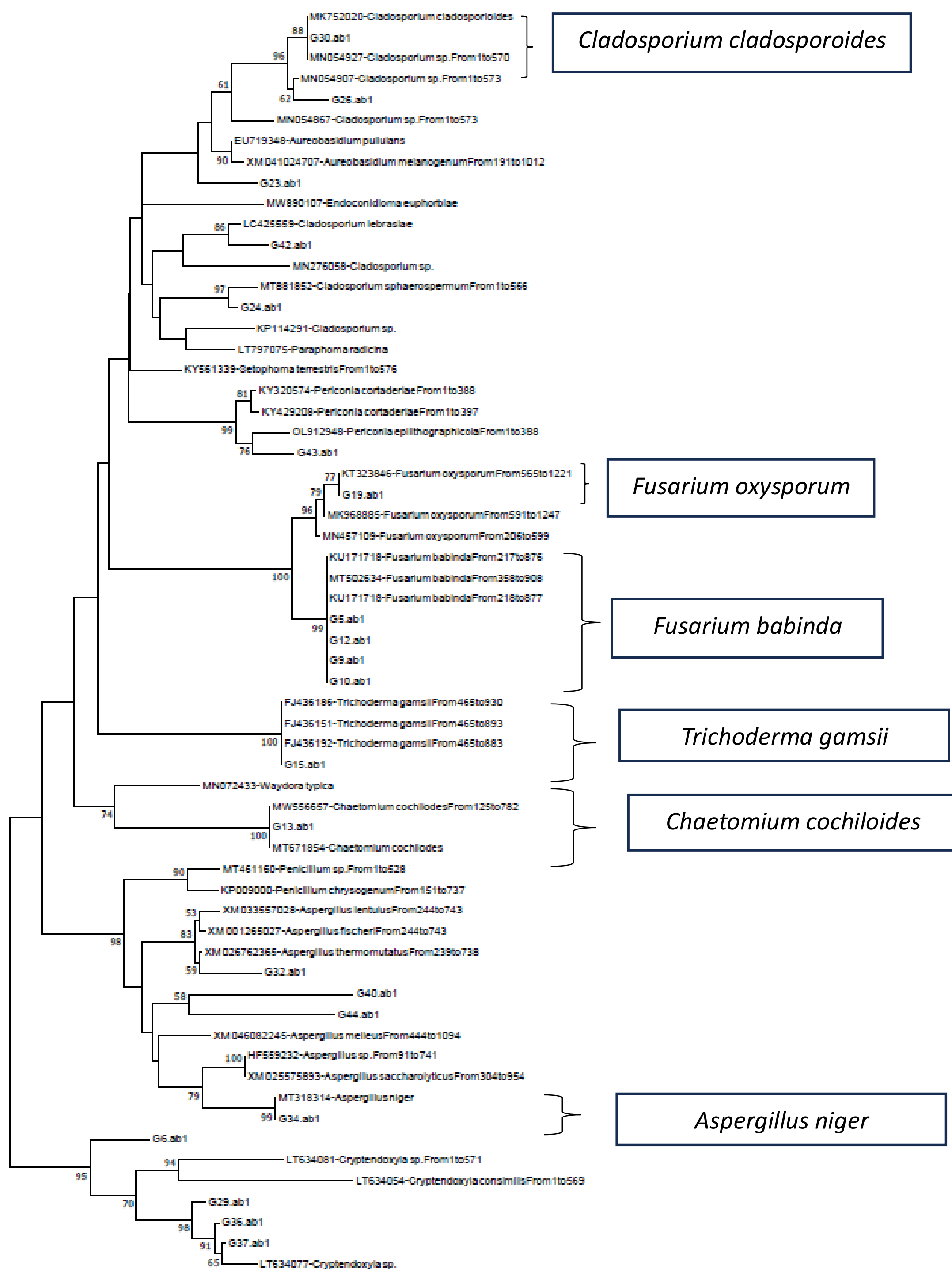

Figure 4: Minimum evolution tree constructed using the Tamura Nei model for the TEF gene region with reference sequences from the BLAST software. Number on branches denotes bootstrap values from minimum evolution analyses.

DISCUSSION

Cultural Identification: Results obtained from cultural identification revealed fungi belonging to three genera: *Fusarium, Aspergillus* and *Penicillium*. Simon *et al*. (2021) worked on the efficiency of indigenous entomopathogenic fungi to control the black bean aphid. He reported some species of *Aspergillus* isolated from the black bean aphid as being entomopathogenic but their potential use to control this aphid was discouraged in the agricultural setting due to their ability to produce toxic secondary metabolites known as aflatoxins that are poisonous to crops and responsible to various health problems such as cancers, immune suppression and growth problems in children in reports of Gong *et al*. (2016). From the findings of Simon *et al*. (2021), it can be explained that the species of *Aspergillus* isolated from potato aphids in our study posed a pathogenic effect on the aphids leading to their death. Seye *et al*. (2014) reported some Aspergillus species (*A. flavus* and *A. clavatus)* in being pathogenic to aphids in laboratory bioassays. This same pathogenic interaction could have also occurred in the field in our studies. Moreover, Alhadidi, (2023) also reported *Penicillium* species extracted from insect cadavers. The cosmopolitan nature of *Penicillium* and its possibility of interactions with insects was mentioned by Visagie *et al*. (2009). He explained that some *Penicillium* species forming associations with arthropods have extended synnema that help in dispersal through the arthropod vectors according to Abbot, (2000) while Roets *et al*. (2007) reported that some form associations with mites simply for the spreading of their spores as such, seen as an opportunistic interaction. *Fusarium* is also a cosmopolitan group of fungi and different studies have confirmed their occasional isolation from insect cadavers (the case in our study) as well as live insects Lav and Guilherminia, (2018). Isolation of *Fusarium* from dead insects has also been reported in literature by, Feng-Yan and Quing-Tao, (1991). They reported 180 *Fusarium* strains isolated from about 150 dead insects.

Molecular Identification using the ITS: The ITS gene region identified six species (*Fusarium oxysporum Aspergillus sydowii, Aspergillus niger, Curvularia affinis, Microascus murinus and Trichoderma erinanaceum*). According to Raja *et al*. (2017), this gene region is the most useful gene (universal barcode) for fungi identification at species level and many environmental surveys have used this gene region to conduct their research. Given the fact that ITS provides greatest probabilities for a varied group of fungi as reported by Schoch *et al*. (2012), species identified based on this gene region are accepted except for specific genera such as *Penicillium, Trichoderma, Aspergillus, Fusarium and Cladosporium* because enough resolution for species identification cannot be done based only on the ITS gene region as mentioned by Raja *et al*. (2017) which was the case observed in our study. This therefore mean that, we can trust the species identified as *Curvularia* and *Microascus* using the ITS gene region. These groups are widely distributed and mainly isolated from the soil, decomposing plant materials and indoor environment according to studies of Sandoval-Denis *et al*. (2016) but few of them are reported to be opportunistic pathogens of insects and animals with humans inclusive. *Curvularia* exhibits a variety of life forms, including saprophytic, endophytic, and pathogenic behaviour on both plants and animals and studies of Isabel *et al*. (2020) reported that the endophytic species have the potential for producing organic compounds that are eco-friendly.

Molecular Identification using the TEF gene region: The TEF gene region could also identify 6 species *Cladosporium cladosporoides, Fusarium oxysporum, Fusarium babinda, Trichoderma gamsii,*

Chaetomium cochiloides and Aspergillus niger. Raja *et al*. (2017) proposed this gene region as a secondary barcode to the ITS gene region for a better resolution to species level in groups belonging to *Cladosporium, Fusarium, Trichoderma and Aspergillus*. Therefore, results based on this gene region can be appreciated. In addition to the species that were identified with the ITS gene, the TEF gene region could identify 3 more species which are *Cladosporium, Trichoderma* and *Chaetomium* that were not possible with ITS gene alone. The association of *Cladosporium* species with insects have been confirmed in many studies confirmed by Nicoletti *et al*. (2024). They have been noted to form associations with beneficial insects as well as pest, including the green peach potato aphid (*Myxus persicae*), the cow pea aphid (*A. craccivora*), the cotton aphid (*A. gossypii*) and the black bean aphid (*A. fabae*) and many species have been reported to be effective in inducing insect pest mortality according to the studies of Nicoletti *et al*. (2024) and have been used as effective biological control agents and effective potato production technologies in past studies performed by Bensaci *et al*. (2022). *Trichoderma* also falls among the cosmopolitan group of fungi that was isolated from aphids in our study. Various species of this fungus have been reported by Hexon *et al*. (2019) for their effectiveness in biocontrol of several insect pests and many plants pathogenic fungi. They interact with insects by producing volatile organic compounds for example 1-octen – 3-ol that attract the insects to their fruiting bodies there by reducing the pest activity on the plant. It has also been reported to have very good potentials in the management of the potato aphid *Marcrosiphum euphorbiae* acting as a pest on tomato plants by the production of volatile organic compound that attract parasitoids and aphid predators as reported by Battaglia *et al*. (2013). According to Seethapathy *et al*. (2023), the genus *Chaetomium* is known for its rapid and saprophytic colonization in a similar manner like *Trichoderma* and it inhabits a wide range of environmental habitats with good biological control potentials in regulation a wide variety of bacteria, fungi as well as insect pests. Moreover, our results can be explained following the studies conducted by Alhadidi, (2003) who reported 30 species of EPF tested on different insects with 16 genera. Similar genera corresponding to his findings were, *Penicillium, Fusarium, Cladosporium, Trichoderma and Chaetomium.* These species were experimentally tested and were noticed to be entomopathogenic. This could support the fact that they must have formed a pathogenic association with the potato aphids, producing spores that grew on the body surface of the aphids and thus leading to their death. This is in accordance with the findings of Abebe *et al*. (2020). Furthermore, Francis *et al*. (2022), reported that some insect pathogenic fungi species may spend their lives within the tissues of the host plant without portraying any symptoms but retain their pathogenic effects against the insects. This could be exactly the case of the fungi species recorded in our findings that led to the killing of the aphids that were cultured. Molecular techniques clarify the doubts, errors and limitations obtained by cultural studies of Paz *et al*. (2011**).** Therefore, the integration of both morphological and molecular approaches permits proper identification of samples than sole dependency on the morphological methods as mentioned in the research of Bich *et al*. (2021).

Phylogenetic relationship based on both ITS and TEF gene regions: Phylogenetic studies using the ITS gene region placed the identified species into five individual clades which are: the *Aspergillus* clade (comprising *A. niger and A. sydowii* sub clades) with a bootstrap support of 67% and 100% support for the subclades respectively, the *Curvularia affinis* clade with a bootstrap support of 100%, *Trichoderma eranaceum* clade with 100% support, *Microascus murinus* with a 100% bootstrap

support and the *Fusarium oxysporum* clade with 93% bootstrap support. Many sequences could not be assigned to a particular group using this gene region due to poor resolution. Therefore, more gene regions are required for a clearer resolution and proper phylogenetic placement of the poorly resolved sequences as mentioned in the studies of by Raja *et al*. (2017). Phylogenetic analyses using the TEF gene region placed the identified samples into five different monophyletic groups made up of *Cladosporium cladosporoides* clade *Fusarium* clade *(F. oxysporum* and *F. babinda* sub-clades), *Trichoderma gamsii* clade*, Chaetomium cochiloides* clade *and Aspergillus clade (A. niger and a polytomous Aspergillus* sub-clade*)* with bootstrap support values of 88%, 100%, 100%, 100% and 79% respectively. *F. oxysporum* formed a sister group with *F. babinda.* This gene region was still not enough to provide enough resolution of all our sequences to species level but has been reported to be widely used in fungal phylogenetic studies according to the works of Stielow *et al*. (2015) and Raja *et al*. (2017). Hence, phylogenetic studies based on the TEF gene region for this study is more informative and accurate than the results obtained with the ITS gene region. This gene region could identify species that were not possible with the ITS gene.

CONCLUSION AND APPLICATION OF RESULTS

Potato aphids are associated with a variety of fungi species. These associations though some posing harm to the aphids in being pathogenic to them, is rather a better strategy that farmers can use as a control measure to combat potato aphids as a component of integrated pest management. Molecular identification is preferred as it could identify many species of fungi compared to identification only by morphological means of which, only 3 species of fungi were identified. The ITS gene region could identify 6 different species (*Fusarium oxysporum, Aspergillus sydowii, Aspergillus niger, Curvularia affinis, Microascus murinus and Trichoderma erinanaceum*) while species identified using the TEF gene regions were also 6 and included *Cladosporium cladosporoides, Fusarium oxysporum, Fusarium babinda, Trichoderma gamsii, Chaetomium cochiloides and Aspergillus niger.* Phylogenetic analysis involved sequences obtained from our study and those from the genbank. We used only two gene regions in our study (ITS and TEF) which posed a limitation in resolution of all our sequences to species level. The ITS gene region is the universal barcode for fungi identification but the TEF gene region is preferable for fungal phylogenetic studies (evolutionary) studies in mycology. Future research should make use of multigene amplifications such as ITS, TEF, RPB2 and LSU for a clearer resolution to species level and a better phylogenetic analysis. Further research is ongoing on multigene identification and phylogenetic study, and to screen entomopathogenic species with bio pesticidal properties against potato aphids which is an environmentally friendly method of pest control compared to synthetic pesticides.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest

REFERENCES

Abebe N, Negeri M, Getu E, Selvaraj T., 2020. Effect of integrating entomopathogenic fungi and botanical extracts against Russian wheat aphid, *Diuraphis noxia* Moedvilko (Hemiptera: Aphididae) in West Showa, Ethopia. *Journal of Entomology and Nematology* 12(1): 18-24.

Abbott SP. 2000. Holomorph studies of the Microascaceae. (Doctoral dissertation). Edmonton: Univ Alberta. 196

Acho, CC., 1998. Human interphase and environmental instability: addressing the environmental problems of rapid urban growth. *Journal of Environment and Urbanization*. 10 (2): 415.

Alhadidi S., 2023. A review of entomopathogenic fungi in Iraq. *Iraqi Journal of Science 1(64): 91- 110.*

Ayonghe SN., 2001. A quantitative evaluation of global warming and precipitation in Cameroon from 1930 to 1995 and projections to 2060: effects on environment and water resources. In: Lambi CM (ed) Environmental issues: problems and prospects. *Unique Printers, Bamenda* 142-155.

Battaglia D, Bossi S, Cascone P, Digilio MC, Duran Prieto J, Fanti P, Guerrieri E, Iodice L, Lingua G, Lorito M, Maffei ME, Massa N, Ruocco M, Sasso R, Trotta V., 2013. Tomato below ground-above ground interactions: *Trichoderma longibrachiatum* affects the performance of *Macrosiphum euphorbiae* and its natural antagonists. *Mol Plant-Microbe Interact* 26:1249–1256.

Bensaci OA, Rouabah K, AliatT, Lombarkia N, Plushikov VG, Kucher DE, Dokukin PA, Temirbekova SK, Rebouh NY., 2022. Biological Pests Management for Sustainable Agriculture: Understanding the Influence of *Cladosporium* Bioformulated Endophytic Fungi Application to Control *Myzus persicae* (Sulzer, 1776) in Potato (*Solanum tuberosum* L.). *Plants 11*, 2055.

Beever RE, Bollard EG. 1970. The nature of the stimulation of fungal growth by potato extract. *Journal of General Microbiology* 60:273-279.

Bich GA, Castrillo ML, Krama FL, Villalba LL, Zapata PD., 2021. Morphological and molecular identification of entomopathogenic fungi from agricultural and forestory crops. *Fleresta e Ambiente* 28(2).

Clifton EH, Jaronski ST, Coates BS, Hodgson EW, Gassmann AJ., 2018. Effects of endophytic entomopathogenic fungi on soybean aphid and identification of *Metarhizium* isolates from agricultural fields. *PLoS ONE* 13(3).

Council Development Plan 2011. *Santa Council* 167.

FAO 2009. Sustainable Potato Production. Guidelines for the developing countries.

Feng-Yan B, Quing-Tao C., (1991). Fusarium species on some insects from china. *Acta Mycol. Sin.* 10:120 –128.

Fogwe ZN., 2014. Montane resources issues in Santa economy of the Western Bamboutos Highlands, Cameroon. *International Journal of Geography and Regional Planning* 1(1): 1-12.

Foncho PA., 1982. The Situation of the Potato Crop in Cameroon and in the World. *Netherlands Potato Consultative* 1(13): 1-12.

Francis F, Fingu-Mabola JC, Fekih IB., 2022. Frederic Francis Direct and Endophytic Effects of Fungal Entomopathogens for Sustainable Aphid Control: A Review. *Agriculture*, *12*(12):2081.

Gong YY, Watson S, Routledge MN., 2016 Aflatoxin exposure andassociated human health effects, a review of

epidemiological studies. *Food Saf* 4:14–27.

Hexon AC, Lourdes M, del-Val E., 2019. Interactions of Trichoderma with Plants, Insects, and Plant Pathogen Microorganisms: *Chemical and Molecular Bases Co-Evolution of Secondary Metabolites* 76887-8.

Hibbett DS, Ohman A, Glotzer D, Nuhn M, Kirk P, Nilsson RH., 2011. Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. *Fungal Biology Reviews* 25(1): 38-47.

Hoggard M, Vesty A, Wong G, Montgomery JM, Fourie C, Douglas RG, Taylor MW., 2018. Characterizing the human mycobiota: a comparison of small subunit rRNA, ITS1, ITS2, and large subunit rRNA genomic targets. *Frontiers in microbiology* 1-*9*.

Humber RA., 2012. Entomophthoromycota: A New Phylum and Reclassification for Entomophthoroid Fungi. *Mycotaxon* **(***120*): 477–492.

Inglis PW, Pappas MCR, Resende LV, Grattapaglia D., 2018. Fast and inexpensive DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. *PLoS ONE* 13(10).

Isabel I, Josepa G, Nathan W, Dana G., 2020. Three *Curvularia* species from clinical and environmental sources. *MycoKeys* 68: 1–21.

Kauserud H, Schumacher T. 2001. Outcrossing or inbreeding: DNA markers provide evidence for type of reproductive mode in *Phellinus nigro limitatus* (Basidiomycota). *Mycological Research* **105**(6), 676-683.

Konje CN, Abdulai AN, Achiri DT, Nsobinenyui DT, Tita MA., 2019. Identification and Management of Pests and Diseases of Garden Crops in Santa, Cameroon. *Journal Of Agriculture and Ecology Research international* 18(2): 1-9.

Lav S, Guilherminia M. 2018. *Fusarium*, an Entomopathogen—A Myth or Reality? *Pathogens* 7 (4):93

Leslie J, Summerell A. 2006. The Fusarium Laboratory manual. United States of America. *Blackwell* 387.

Machado-Assefh CR, Said-Adamo M M, Cortéz SD, Gialdi AIL, Isasmendi GL, Jaime Ortego J, Alvarez AE., 2023. Newly recorded plant-aphid associations: Implications for PLRV and PVY control in potato crops. *Crop protection* 167:106-202.

Membang G, Ambang Z, Mahot HC, Kuate AF, Fiaboe KKM, Hanna R., 2021. Thermal response and horizontal transmission of cameroonian isolates of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* e Candidates for microbial controls of the banana root borer Cosmopolites sordidus. *Fungal Ecology* 50:101-042

Mengui KC, Oh S, Lee HS., 2019. The technical efficiency of smallholder irish potato Producers in Santa Subdivision, Cameroon. *Agriculture* (9):259.

Mekwe A, Ulrichs C, Nana P, Akutse KS, Fiaboe KKM, Maniania NK, Ekesi S., 2018. Evaluation of the Entomopathogenic Fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Isaria sp.* for the Management of Aphis craccivora (Hemiptera: Aphididdae). *Journal of Economic Entomology* (111): 1587-1594.

Ndikintum NF., 2009. The role of night paddock manuring in the reduction of poverty and conflict amongst farmers and grazers in Small Babanki

(Cameroon). *Master's in philosophy Thesis* 142.

Neba AS., 1999. Morden Geography of republic of Cameroon. *Neba publisher, Bamenda Cameroon.*

Nicoletti R, Russo E, Bechhhimani A., 2024. Cladosporium-insect relationships. J. Fungi 10,78.

Njomo LM, Arnolds C, Ngek PS., 2019**.** Enhancing Indigenous Agricultural Management Techniques: A Framework to Minimize Post-harvest Losses in Potato Farming in Bui Division, Northwest Region of Cameroon. *International Journal of Management and Humanities* (3): 2394-0913.

Nouh FAA., 2019. Endophytic fungi for sustainable agriculture. *Microbial biosystems* 4(1):33-44.

Parfait NG, Didier NR, Fosah MR, Teke AN, Delphin KA., 2023. Influence of some abiotic factors on the diatom densities in the mezam river (Bamenda, North-west Cameroon). *African Journal of Biology and Medical Research* 3(6): 40-55).

Paz A, González M, Crawford A, Código de barras de la vida., 2011. Introducción y perspectiva. *Acta Biológica Colombiana* 16: 161-176.

Quecine MC, Bini AP, Romagnoli ER, Andreote FD, Moon DH, Labate CA., 2014. Genetic variability in Puccinia psidii populations as revealed by PCR-DGGE and T-RFLP markers. *Plant disease* 98(1):16-23.

Raja HA, Miller AN, Pearce CJ, Oberlies NH., 2017. Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community. *Journal of Natural Products* 80: 756−770.

Roets F, Wingfield MJ, Crous PW, Dreyer LL., 2007. Discovery of fungus-mite mutualism in a unique niche. *Environ Entomol* 36:1226–1237.

Sandoval-Denis M, Gené J, Sutton DA, Cano-Lira JF, De Hoog GS, Decock CA, Wiederhold NP, Guarro J., 2016. Redefining Microascus, Scopulariopsis and allied genera. *Persoonia - Molecular Phylogeny and Evolution of Fungi* 36:1-36.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. 2012. Fungal Barcoding Consortium; Fungal Barcoding Consortium Author List. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A. 109(16):6241-6.

Scorcetti AC, Humber RA, García JJ, Lastra CCL., 2007. Natural Occurrence of Entomopathogenic Fungi (Zygomycetes: Entomophthorales) of Aphid (Hemiptera: Aphididae) Pests of Horticultural Crops in Argentina. *BioControl 52*: 641–655.

Seethapathy P, Sankarasubramanian H, Lingan R, Thiruvengadam R., 2023. *Chaetomium* Sp.: An insight into its atagonistic mechanisms, mass multiplication and production cost analysis. In: Amaresan, N., Dharumadurai, D., Babalola, O.O. (eds) Agricultural Microbiology Based Entrepreneurship: *Microorganisms for sustainability* (39):978-981.

Seye F, Bawin T, Boukraa S, Zimmer JY, Ndiaye M, Delvigne F, Francis F., 2014. Effect of entomopathogenic Aspergillus strains against the pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae). *Appl Entomol Zool* 49: 453–458.

Simon BB., Robert AM, Nickson M, Paola S, Thibault N., 2021. Efficacy of indigenous entomopathogenic fungi against the black aphid, Aphis fabae

Scopoli under controlled conditions in Tanzania. *International Journal of Tropical Insect Science* 41:1643–1651.

Stielow J, Lévesque C, Seifert K, Meyer W, Irinyi L, Smits D, Renfur R, Verkley G, Groenewald M, Chaduli D., 2015 One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia*, *35*, 242-263.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739

Tian L, Hu B, Zhou H, Zhang W, Qu L, Chen Y., 2010. Molecular phylogeny of the entomopathogenic fungi of the genus Cordyceps (Ascomycota:Clavicipitaceeand its evolutionary implication. *Journal of Systematics and Evolution* 48(6): 435–444.

Ullah S, Raza MBA, Alkafafy M, Sayed S, Hamid IM, Majeed ZM, Riaz AM, Gaber MN, Asim M., 2022. Isolation, identification and virulllence of indigenous entomopathogenic fungal strains against the peach-potato aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), and the fall armyworm, Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control* 32:2.

Visagie CM, Roets F, Jacobs K., 2009. A new species of Penicillium, *P. ramulosum* sp. nov., from the natural environment. *Mycologia* 101(6): 888–895.

Vu VH, Hong S, Kim K., 2007. Selection of Entomopathogenic Fungi for Aphid Control. *Journal of Bioscience and Bioengineering* 104(6):498–505.

Yi X, Gray SM., 2020. Aphids and their transmitted potato viruses: A continuous challenges in potato crop. *Journal of Integrative Agriculture,* 19(2): 367-375.