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Identification of fungal species isolated from diseased cabbages in the western highlands of Cameroon

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ABSTRACT

Some fungal species of cabbage are pathogenic contributing significantly to pre-harvest and post-harvest loses leading to food insecurity. Therefore, a study was carried out to identify fungal species on diseased cabbage in the Western highlands of Cameroon. One hundred samples of diseased cabbage were collected from Santa and Dschang. Fungal species was isolated from the leaves, and cultural and molecular identification of the Internal Transcript Spacer (ITS) and Translation Elongation Factor (TEF) gene regions was done. The results of cultural study indicated that out of 100 samples, 81 were infected with fungi species. The results of molecular identification of fungal isolates base on ITS regions revealed that a total of 45 fungal species belonging to 12 genera with *Trichoderma* being the highest with 16 isolates followed by *Fusarium* with 10 isolates. The fungal species identified from TEF regions showed that 51 species of fungal species were isolated from cabbage belonging to 8 genera with *Trichoderma* the most dominant (26 species) closely followed by *Fusarium* (16 species). The results clearly revealed that molecular identification was more accurate in identifying fungi species than the cultural method. The identified fungal species will be used in pathogenicity test to know the actual pathogens of cabbage and to devise appropriate control measures.

Keywords: Vegetable, Fungal-taxonomy, Species-richness, Isolation, Cultural, Molecular.

INTRODUCTION

A vast agricultural potential exists in Cameroon, which could make a substantial contribution to the country's efforts to minimized poverty and encourage sustainable development (Asongwe et al., 2014). Agricultural production accounts for 50% of total exports and above 75% of the active population of Cameroon is involved in agriculture (Gbetnkom and Khan, 2020). Agriculture has been a crucial industry that has greatly boosted the country's economy for a number of decades. About 42.4% of the country's GNP was generated by agriculture in 1999 (Gbetnkom and Khan, 2020). Recently, the Cameroonian government has placed a strong emphasis on agriculture as one of the foundations for a developing nation.

Brassica oleracea L. commonly called cabbage is a member of the Brassicaceae family, which also includes mustard. Other cruciferous vegetables, such as broccoli,

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collard greens, brussels sprouts, kohlrabi, and sprouting broccoli, are regarded as cultivars of *Brassica oleracea* (Plants of the World Online, 2021). Cabbage is useful to man and animals and is consumed more as food, eaten in vegetable salads and as vegetable meals (Alexandra et al., 2020). Cabbages are prepared in many ways for eating. Cabbage can be pickled, fermented for dishes such as sauerkraut, steamed, stewed, sautéd, braised, or eaten raw. Cabbage is a good source of vitamin K, vitamin C and dietary fiber (Morab et al., 2020). According to Sharma et al. (2018), Brassicaceae family which includes cabbage and other cruciferous vegetables, has recently come to be recognized as a significant dietary source of chemoprotective phytochemicals.

Commercial production of cabbage crop is varies among regions, with the plant presenting well-drained sandy loam and sandy soil with pH range from 6.2 to 6.5. In Cameroon it can grow in all regions (Sharma et al., 2018). The production of cabbages in Cameroon was 73.7 tonnes in 2019 and is forecast to change by an average of 2.9%. The country had approximately 2.4 hectares under cabbages cultivation (Abia et al., 2016). In 2019 Cameroon exported 89 tonnes of cabbages. For the year 2019 alone, the demand for Cameroon cabbages (vegetables category) had gone up, changing by 394.4 percent compared to the year 2018. Between 2017 and 2019, cabbages' exports increased by 345% netting US$0.08m for the year 2019.This low production is due to several factors such as lack of improved raised beds, temperature, day length and several diseases.

Cabbage is attacked by a large number of pathogens that is, fungi, bacteria, viruses and nematodes (Sharma et al., 2018). It is estimated that over 30 – 40% of crop losses may be with these diseases (Chai et al., 2022). Cabbage wilt or yellowing is caused by *Fusarium oxysporum* (Mehraj et al., 2020) and it is an important cabbage pathogen. Downy Mildew is airborne fungal disease; the pathogen belongs to oomycetes that need moisture in soil with low temperature which also attacks cabbage in Cameroon (Thines and Choi, 2016). Some of these diseases in Cameroon and in Santa are the club root of cabbage, black leg, leaf spot, Blight and Black rot (Ngangjoh, 201). Some fungi species reported to have been isolated from cabbages are *Alternaria brassicocola* (Iacomi-Vasilescu et al., 2004), *Fusarium oxysporum* (Mehraj et al., 2020), *Aspergillus* (Li et al., 2023), *Plasmodiophora brassicae* (Hwang et al., 2012) often identified from its root deformation nurture which looks like ginger, *Hyaloperonospora parasitica* causes downy mildew in cabbage (Saha et al., 2020).

Neopseudocercosporella capsellae (white leaf spot disease) is an important pathogen in crucifers leading to enormous yield loss under cool and wet conditions (Gunasinghe et al., 2016). *Fusarium* is often noticed for its role in causing wilting or yellowing of cabbage leaves in the field, (Mehraj et al., 2020). The symptoms of *Alternaria* infection in the field are chlorosis and necrosis of leaf tissues (Rahimloo and Ghosta, 2015), *Verticillium* causes yellowing in Chinese cabbage (Narisawa et al., 2004). Cultural character is the most use form of fungal pathogen identification in Cabbage base on the morphology of the culture with focused on the colour both in front and reverse, the colony margin, the colony diameter (Shim et al., 2013). Molecular techniques of identification provide alternative methods for taxonomic studies and are important tools in solving the problems of species delimitation (Huang et al., 2013). Molecular identification of plant has been of greater application than any method of species identification (Singh et al., 2023).

According to Nadeem et al. (2018), the cabbage plant's root and leaf systems are frequently affected by fungi that cause plant physiology disruptions by harming the vascular system and delaying photosynthesis, which lowers the yield and quality of the crop. However, the climatic conditions of the western highlands of Cameroon favours the growth of cabbage. The aim of the study was to identify fungal species isolated from diseased cabbages in the western highlands of Cameroon.

MATERIALS AND METHODS

Description of Study Site

The study was carried out in Santa and Dschang. Santa Sub-divisions is found in Mezam Division of the North West Region of Cameroon. It is located between latitudes 5°42′and 5°53′ north of the equator and longitudes 9°58′ and 10°18′ east of the Greenwich Meridian. The population of Santa in 1987 was estimated to about 57,477, and it was projected in 2015 to have population of 319,870 (Santa Council Development Plan, 2014) and 90% of this population are engaged in farming and grazing. Santa covers a surface area of about 532.67 km². It is bordered to the north by Bamenda sub division, west by Bali and Batibo sub-divisions, south by Wabane, Babadjou and Mbouda and the east by Galim. The mean annual temperature of the area varies from 21.8 to 30.8° C. Its annual rainfall is between 2000-3000 mm and the rainy season starts from March to September and the dry season from October to February. The soils in this area are fertile and support a large human population. The altitudinal range is from 600 to 2600 m, making this highland favourable for animal rearing, crop and vegetable production in the Western Highlands of Cameroon. It is located about 20 km from Bamenda. Santa is cut across by the Bamenda-Bafoussam highway running through it and serves also as the main route of evacuation of the farm produce from this area (Konje et al., 2019).

Dschang was the second site selected for the study and is located in the western region, in West Cameroon (Figure 1). Dschang is situated at an altitude of approximately 1407M above sea between latitude 5°20′ north and longitude 10°30′ west (Kouam et al., 2018). This city, which has a total area of 225 km2, has a cold, mild climate (wet tropical), similar to that of Equatorial Guinea, with two distinct seasons: the rainy season, which lasts from mid-March to mid-October, and the dry season, which lasts from November to February. Rainfall has an annual height of 1809 mm and is unimodal (Fink et al., 2017). August and September see the most precipitation. Ferralitic and hydromorphic soils are found in this city. The map of the study area is presented in Figure 1.

Fungal Collection and Isolation

A total of 10 farms were selected in both Santa and Dschang in the Western highlands of Cameroon. Each of the sampled farm measured at least a hectare in dimension. The selected farms were then survey of the presence of fungal diseases of cabbage**.** A total of five cabbage leave was collected per cabbage farm for any cabbage whose leaves showed fungal diseases symptoms from the selected 10 farms in Santa and Dschang each making a total of 100 samples. Each sample was immediately stored in a ziplock bag and labeled. These samples were put in coolers and transported by means of public transport to the Life Science Laboratory in the University of Buea for isolation and morphological identification of pathogenic fungi.

Potato Dextrose Agar (PDA) was used for isolation of fungi. In the Laboratory, the working environment was surface sterilized with 70% alcohol to reduce chances of contamination. PDA was prepared according to manufacturer's instructions (Kinge et al., 2023).

Isolation of fungal species from leaves portion

About 5 mm of the infected part of cabbage leaves were cut with the use of sterile razorblade and the portions were put in small nets, surface sterilized by immersing in 10% sodium hypochlorite solution for 3 minutes and rinsed in distilled water, followed by rinsing with 70% alcohol for 1 minute. They were then rinsed in sterile distilled water and finally tap water, then plated on the solidified PDA medium in labeled Petri dishes. The plates were sealed with parafilm wax and incubated at room temperature (25°C) in the dark for 7 days. After 7 days, fungi that grew on the petri dishes were sub-cultured on fresh PDA plates at room temperature. This was according to the protocol of Leslie and Summerrel, (2008). For

the isolation of pure culture of fungal isolates, the isolated fungal cultures were transferred into fresh pre-sterilized Petri plates and incubated until fungal colonies appeared. The separated colonies were subculture on PDA Petri plates to obtain the pure fungal culture. The fungal colonies obtained were then identified.

Cultural and morphological identification

Cultural features such as colony diameter, colony colour, texture, margin, form, elevation was recorded as from the 3rd day after cultured to the 7th day at the live science laboratory of the University of Buea by observing the growth forms on PDA physically. The mycelium was observed under a light microscope at a magnification of 20 and 40 to view the spore nature, anatomy and crosschecked with those in literature.

Genomic DNA extraction, amplification, and sequencing

The mycelia of these pure cultures were harvested and stored in 10% glycerol in eppendorf tubes and 2 mL screw cabs for subsequent molecular identification. Genomic DNA was extracted from cultures following the Sorbitol-CTAB protocol according to Inglis et al. (2018). DNA concentrations and purity were determined with a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Working DNA concentrations for polymerase chain reactions (PCRs) were obtained by adjusting the initial concentrations of the extracted genomic DNA to 100 ng/µL. 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). 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 electrophoresed on 0.8% agarose gel after staining with ethidium bromide run in 0.5 X TBE buffer at 85 V for 40 min and documenting under 100% UV light with a gel transilluminator (MWG-BIOTECH). The amplified PCR products were purified using PCR clean with beads according to the manufacturer's instructions (Arbeli and Fuentes, 2007).

DNA sequence identification

Preliminary identification based on BLASTn searches in NCBI of ITS and TEF sequences against those of reference sequences in GenBank was made to confirm the sequences of the fungal isolates from cabbages. These sequences were blasted and compared along with those similar in NCBI (GenBank).

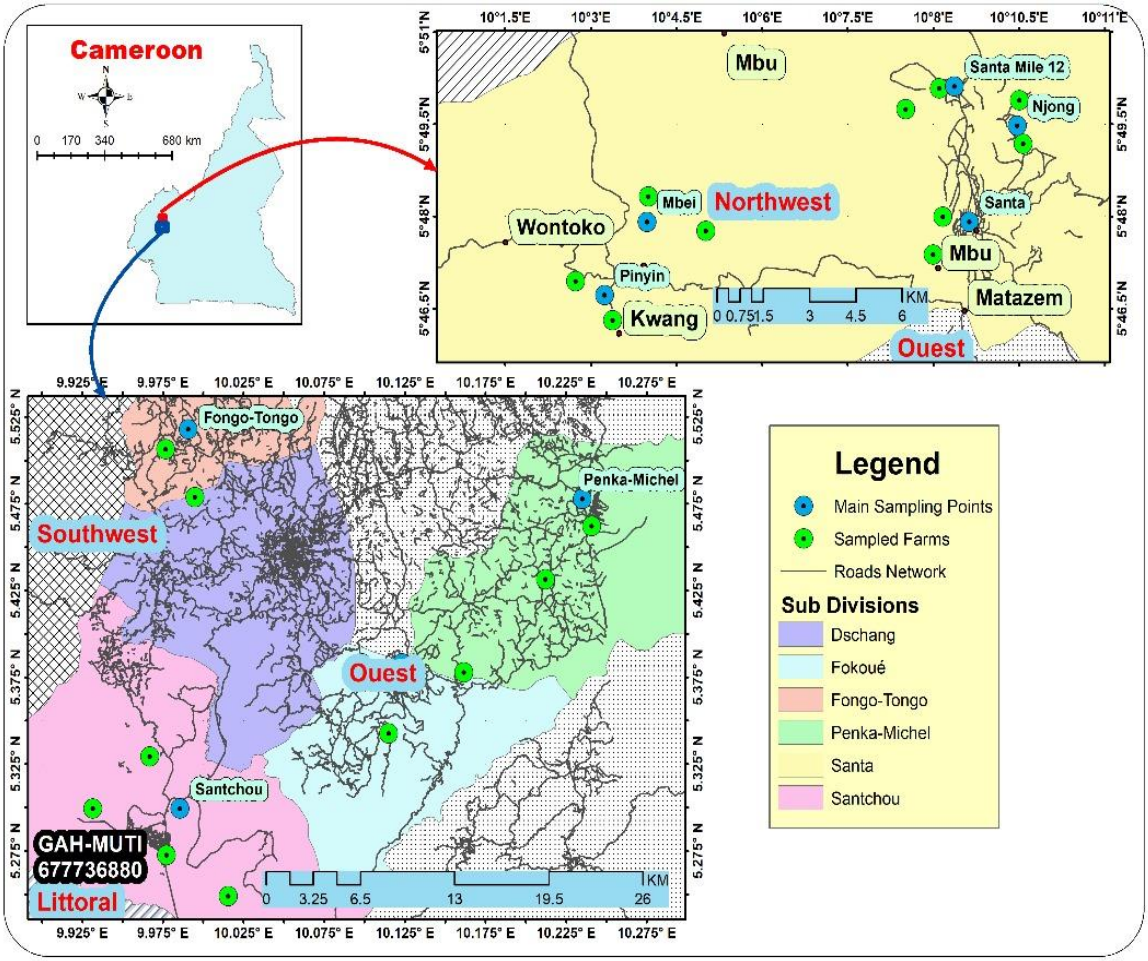

Figure 1: Map of study Area (GAH-MUTI, 2024).

RESULTS

Field Symptoms of Fungi Diseases of Cabbage

The leaves of some infected cabbage plants showed signs of withering. The outer leaves of most cabbage in Santa and Dschang began to wilt before moving inward to the interior leaves. Additionally, some of the infected cabbage leaves were becoming brown or yellow at the leaf margins or the entire leaf. Some cabbages showed signs of retarded growth and had a stunted appearance, which had an impact on the crop's output. On some cabbage leaves, there were necrotic lesions or dead regions. These lesions typically begin tiny and grow to become brown patches. Additionally, vascular tissue darkening was a common occurrence in many cabbage plants. In some cases, there was fall of premature leaves.

Additionally, some cabbage leaves, particularly in Dschang, had small to big circular or irregularly shaped spots on them. Typically, these lesions on the leaves begin as small, dark brown or black dots and extend as days progressed. In certain cases, the lesions in the Dschang and Santa cabbage fields observed had concentric ring pattern with a lighter center and a darker margin. The majority of cabbage leaves that had numerous spots on them became paler, showing evidence of poor photosynthesis. Also, some infections showed

symptoms of soft rot on the cabbage leaves. The affected tissues became water-soaked, mushy, and decayed attracting flies on the cabbage leaves (Figure 2).

Cultural characteristic of isolated fungi species

The results of the study on the cultural characteristics of fungal diseases of cabbage provide important insights into the prevalence and characteristics of fungal pathogens affecting cabbage plants. Out of the 100 samples that were cultured, a significant majority (81 samples) were found to be attacked by fungal pathogens.

The results on morphological characteristics revealed diverse characteristics among the fungal species identified. Some samples exhibited circular growth margins with an ash surface colony color, while others displayed a raised growing form with circular surfaces and green or dark-colored reverses. Additionally, there were species with floppy white colonies that appeared creamy when the petri dish was turned over. Furthermore, many of the species exhibited irregular growing margins and a variety of colony colors, including green, white, leafy green, pink, and orange. The growth parameters recorded were as follows, 6 samples had circular growth margins with an ash surface colony colour and looking dark in their reverse's colony colour. Ten samples had a raised growing form with circular surfaces and were looking green and dark in the reverse colony colour. Other species were floppy white in colour and creamy when the petri dish was turn on the reversed side. Many of the species also show irregular growing margins and colony. Colony colours like green, white, white, green, leafy green, pink and oranges were observed. Out of the 100 samples collected 81 of the samples proved to be infected with fungi diseases with variable cultural characters as presented in Table 1.

These findings suggest a significant diversity and range of fungal pathogens affecting cabbage plants. The variations in colony characteristics and colors indicate the presence of multiple fungal species with distinct morphological features. Figure 3 presents plant pathogenic fungi that were isolated in the laboratory using PDA. This information can aid in the identification and classification of the fungal pathogens responsible for cabbage diseases (Figure 3).

Molecular of Identification Fungal species associated with diseased cabbage

The results based on BLASTn in NCBI showed that 7 subspecies of *Fusarium oxysporum* were identified belonging to the order Hypocreales and family Nectriaceae. This was the second most abundant pathogen that was isolated and only two subspecies of *Fusarium graminearum* were obtained while a single subspecies of *Fusarium caucasicum* was obtained. The most abundant fungi pathogen identified was *Trichoderma harzianum* with 13 strains belonging to the order Hypocreales and family Hypocreaceae. Other species *Trichoderma* sp (6 species), *Trichoderma breve* (1 strain), *Trichoderma lixii* (1 strain) were identified indicating that the genus was the most abundant in the samples. Four strains of *Curvularia senegalensis* were identified belonging to the order Pleosporales and family Pleosporaceae. The genus *Alternaria* had three species which included *Alternaria* sp. *Alternaria alternate* and *Alternaria tenuissima* belonging to the order Pleosporales and family Pleosporaceae. The genus *Penicillium* had only a single species which was *Penicillium citrinum* from the order Eurotiales and family Trichocomaceae. Another species which was identified that belonged to the order Eurotiales and family Aspergillaceae was *Aspergillus niger.* Three species were identified which belonging to the order Pleosporales and family Didymellaceae. These three species were *Phoma* sp, *Dothideomycete* sp. and *Epicoccum nigrum. Curvularia pseudobrachyspora* was identified which belonged to the family Pleosporaceae and order Pleosporales. Lastly, *Candida railenensis* and *Meyerozyma caribbica* were identified and these were the two species of yeast that affected cabbage in the field as seen in Table 2.

From the results in Table 2, it can be seen that the pathogens were identified base on

the percentage of their query cover. A total of 45 species of plant pathogenic fungi were isolated from cabbage samples. The pathogens were divided into 12 genera dominated by the order Hypocreales which had species in the genera *Fusarium, Trichoderma* followed by Pleosporales which had species in the genera *Phoma, Alternaria and Penicillium*. It was found that *Trichoderma* is the genera highly responsible for cabbage disease in the Western highlands of Cameroon followed by *Fusarium*. This can be seen in Figure 4.

Identification Comparisons from ITS and TEF DNA Sequences

Results of BLASTn searches based on ITS revealed that 36 isolates had a high level of DNA sequence similarity base on their query cover which was 98–100% with *Trichoderma* from ITS while for the TEF region it ranged from 57-99%. Also, comparing the query for *Fusarium* species from ITS region it was realized that it ranges between 81-100% and for TEF region, Fusarium species query cover was between 46-99%. A general comparison on the ITS and TEF sequences showed that the ITS sequences had the highest percentages of match as compared to the TEF sequences as demonstrated by the results. The percentage identity for ITS sequences was lower than that of the TEF sequences as the percentage identity of ITS sequences ranged between 83-99% while TEF sequences ranged between 88-99%.

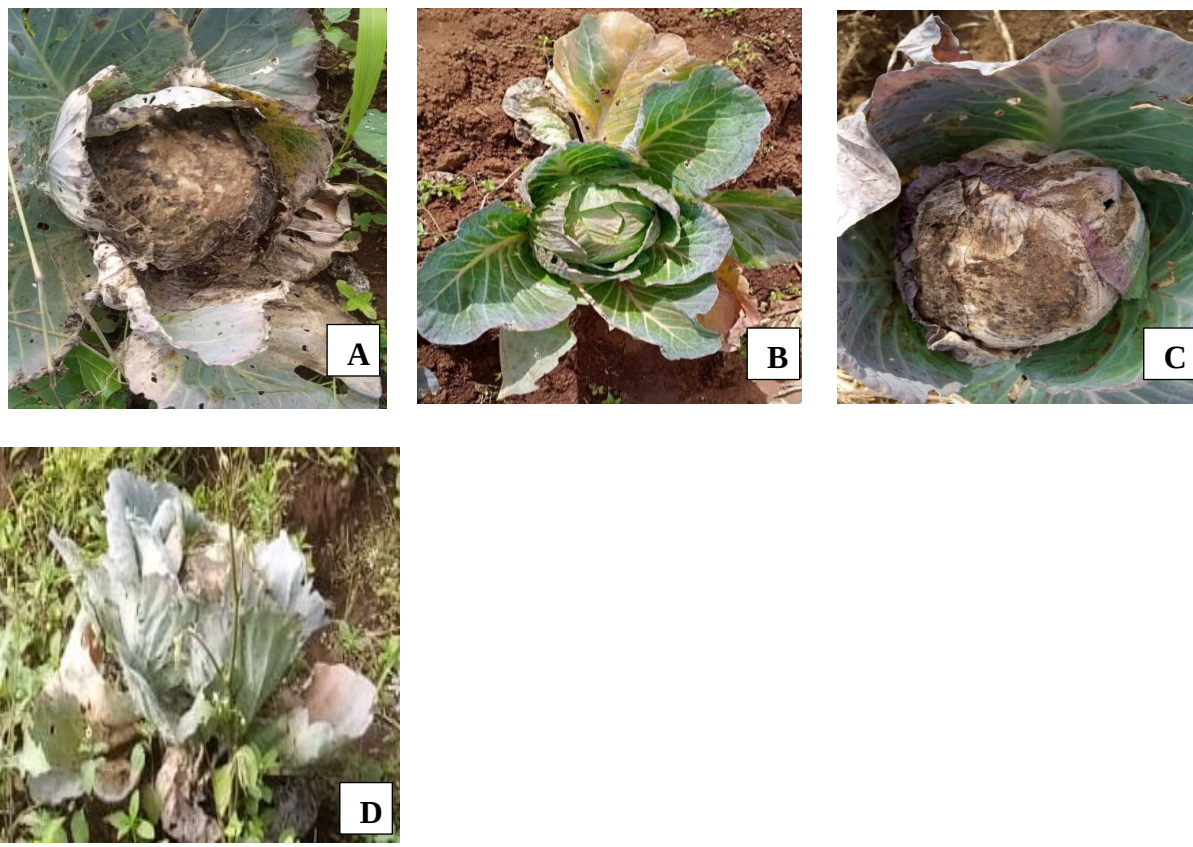

Figure 2: Most prevalence symptoms of cabbage observed in the farms in Santa and Dschang. A) Dark and yellow sports on the leaves with a rottened head, B) Yellowing of leaves, C) Leaves looking purple like with a try head D) Wilting and yellowing of leaves.

Table 1: Different Cultural Characters from Fungi species isolated from cabbages.

Isolates grouped with similar characters	Suspected Fungi species	Colony Form	Colony Margin	Surface Colony Color	Reverse Colony Color
F48, F49, F50, F51, F70, F78	*Aspergilus* sp	Raised	Circular	Ash	Dark
F54, F59, F9, F15	*Aspergilus* sp	Raised	Irregular	Green	Dark
F1	*Penicillium* sp	Raised	Irregular	Pink	Pink
F2, F11, F46, F56, F68	*Penicillium* sp	Raised	Irregular	Green	Dark
F3,F64	*Fusarium* sp	Raised	Irregular	Fluufy white	Cream
F4,F22,, F34, F65	*Fusarium* sp	Raised	Irregular	Cream white	Brown
F5, F29, F37, F38	*Fusarium* sp	Raised	Irregular	Cream	Brown
F6,F33, F47	*Fusarium* sp	Raised	Irregular	Cream	Violet
F7,F40, F66	*Aspergillus* sp	Raised	Circular	Black cream	Black
F12, F32,F39, F77	*Penicillium* sp	Raised	Irregular	Ash	Dark
F31, F53	*Fusarium* sp	Umbonate	Irregular	White	Pink
F14, F26, F45, F58, F19, F23	*Penicillium* sp		Irregular	White green	Dark
F17, F44	*Penicillium* sp	Flat	Irregular	Green	Black
F18, F28, F13	*Aspergillus* sp	Umbonate	Irregular	White green	Green
F20, F30	*Penicillium* sp	Raised	Irregular	Green	Leafy Green
F35, F21, F36, F73	*Aspergillus* sp	Raised	Circular	Fluffy white	Creamy
F24, F25, F43,F55	*Penicillium* sp	Raised	Irregular	Leafy green	Cream white
F27, F71	*Fusarium* sp	Umbonate	Circular	White	Violet
F42	*Alternaria* sp	Raised	Irregular	Dark	Dark
F60	*Penicillium* sp	Raised	Irregular	White	Yellow
F62	*Alternaria* sp	Raised	Circular	Pink	Brown
F63	*Alternaria* sp	Raised	Circular	White	Orange
F69	*Penicillium* sp	Umbonate	Irregular	Fluffy white	Orange
F75	*Fusarium* sp	Raised	Circular	Pink	Pink
F76	*Fusarium* sp	Raised	Irregular	Orange	Redish
F81	*Aspergillus* sp	Flat	Irregular	Black/ white	Black

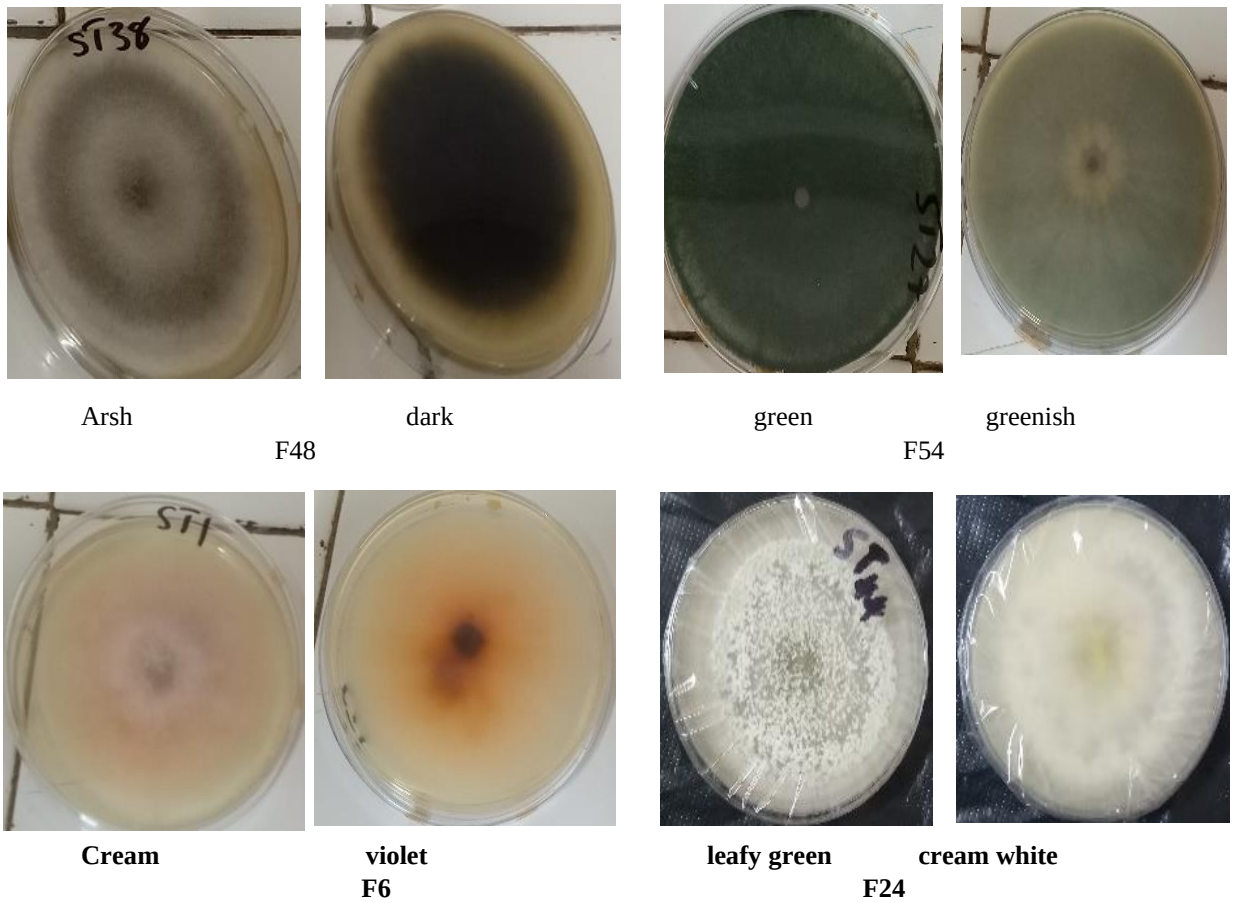

Figure 3: Cultural Characters Observed from different Cabbages.

Table 2: Molecular Identification of Fungal Pathogens from NCBI.

Sample Number	Scientific name	Max scores	Total scores	Query cover	E value	Per. Ident	Acc. len	Accession
F1	*Fusarium oxysporum*	1000	1000	98%	0.0	99.64%	1135	MT453296.1
F2	*Trichoderma harzianum*	1129	1129	98%	0.0	99.36%	1708	KC330218.1
F5	*Trichoderma harzianum*	1062	1062	99%	0.0	96.78%	656	MF782822.1
F9	*Trichoderma harzianum*	1112	1112	98%	0.0	99.35%	656	MF782822.1
F10	*Trichoderma harzianum*	1133	1133	98%	0.0	99.52%	656	MF782822.1
F11	*Candida railenensis*	998	998	98%	0.0	94.63%	640	FM178302.1
F13	*Fusarium oxysporum*	987	987	86%	0.0	98.91%	1135	MT453296.1
F14	*Trichoderma harzianum*	1142	1142	98%	0.0	99.68%	656	MF782822.1
F17	*Trichoderma harzianum*	1134	1134	97%	0.0	99.68%	676	EF432289.1

F18	*Trichoderma* sp	1105	1105	98%	0.0	98.09%	991	MF076625.1
F19	*Trichoderma breve*	1138	1138	99%	0.0	99.21%	640	MN400089.1
F20	*Meyerozyma caribbica*	1059	1059	97%	0.0	97.07%	951	KP132413.1
F23	*Trichoderma harzianum*	1133	1133	99%	0.0	99.05%	656	MF782822.1
F24	*Trichoderma* sp	1138	1138	97%	0.0	99.68%	991	MF076625.1
F27	*Fusarium graminearum*	1000	1132	100%	0.0	98.75%	570	OL364745
F28	*Trichoderma harzianum*	1142	1142	100%	0.0	99.21%	656	MF782822.1
F31	*Fusarium oxysporum*	994	994	85%	0.0	98.40%	573	MK910069.1
F32	*Trichoderma lixii*	1098	1098	99%	0.0	97.95%	646	AY605743.1
F35	*Fusarium oxysporum*	1005	1005	100%	0.0	98.93%	1135	MT453296.1
F38	*Penicillium citrinum*	1024	1024	98%	0.0	99.64%	846	MN788102.1
F40	*curvularia pseudobrachyspora*	972	972	96%	0.0	97.37%	583	NR_164423.1
F42	*Trichoderma gamsii*	1105	1105	100%	0.0	98.87%	857	KM491887.1
F44	*Aspergillus niger*	1107	1107	98%	0.0	99.51%	613	MN100313.1
F45	*Trichoderma harzianum*	1140	1140	100%	0.0	99.06%	1708	KC330218.1
F48	*Phoma* sp	998	998	98%	0.0	100.00%	556	JQ388278.1
F49	*Curvularia senegalensis*	1005	1005	100%	0.0	98.93%	1089	MT476858.1
F50	*Fusarium caucasicum*	977	977	100%	0.0	98.05%	1404	LR583698.1
F51	*Curvularia senegalensis*	1009	1009	98%	0.0	99.64%	1089	MT476858.1
F54	*Trichoderma* sp	1109	1109	100%	0.0	99.03%	632	MK870992.1
F55	*Trichoderma harzianum*	1146	1146	98%	0.0	99.84%	656	MF782822.1
F56	*Trichoderma harzianum*	1140	1140	98%	0.0	99.68%	656	MF782822.1
F57	*Trichoderma harzianum*	1142	1142	100%	0.0	99.21%	656	MF782822.1
F58	*Trichoderma Sp*	1142	1142	98%	0.0	99.68%	991	MF076625.1
F59	*Trichoderma harzianum*	1149	1149	100%	0.0	99.37%	656	MF782822.1
F60	*Curvularia senegalensis*	1009	1009	91%	0.0	99.28%	1089	MT476858.1
F61	*Fusarium graminearum*	652	726	91%	0.0	88.04%	574	MK079935.1
F67	*Curvularia senegalensis*	987	987	87%	0.0	98.73%	1089	MT476858.1
F68	*Trichoderma* sp	1138	1138	99%	0.0	99.21%	991	MF076625.1
F69	*Fusarium oxysporum*	1000	1000	81%	0.0	99.28%	571	MN219649.1
F70	*Alternaria* sp	977	977	96%	0.0	97.40%	1113	MH102088.1
F73	*Dothideomycete* sp	987	987	87%	0.0	99.09%	577	EU680546.1
F74	*Alternaria alternate*	891	891	98%	0.0	94.64%	597	MF575850.1
F75	*Fusarium oxysporum*	990	990	99%	0.0	98.39%	573	MK910069.1
F76	*Fusarium oxysporum*	1009	1009	99%	0.0	99.11%	573	MK910069.1
F77	*Alternaria tenuissima*	1064	1064	100%	0.0	99.49%	596	MK972908.1
F81	*Epicoccum nigrum*	931	931	100%	0.0	96.27%	787	MH931271.1

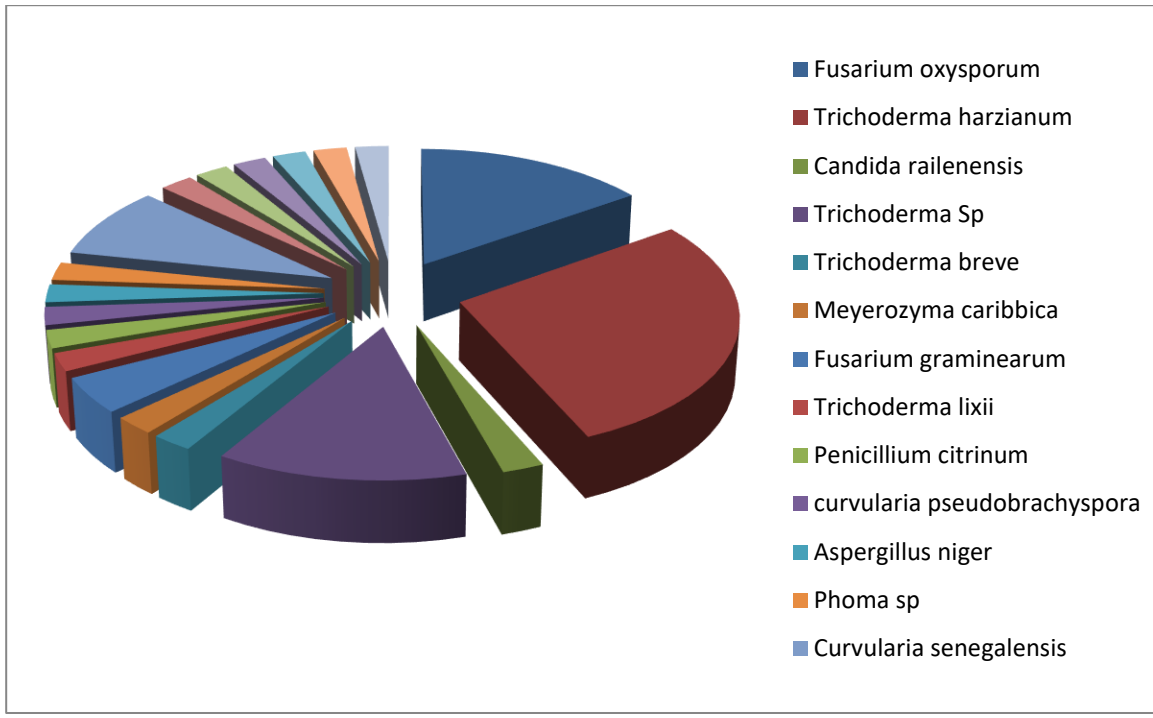

Figure 4: Species richness of fungi pathogens isolated from diseased cabbage.

DISCUSSION

Signs and symptoms for *Fusarium* sp. on cabbage were yellowing of leaves, circular brown spots and leaves getting dry after severe conditions. These symptoms were in line with that of Okungbowa and Shittu (2012) who conducted an overview on *Fusarium* wilts and revealed that Black leg causes dark, sunken cankers at the base of the stem or light brown circular leaf spots were caused by *Fusarium* sp. These results are also supported by Claassen (2016) who reported that black leg diseases cause by *Phoma lingam* results to Cankers, causing wilting, stunting, and cabbage plant death. According to Azevedo et al. (2000) *Alternaria* is responsible for leaf spot diseases of cabbage. This supports the results obtained in the present study because the cabbage leaves with leaf spot disease were identified to be caused by *Alternaria*. Infected cabbage plants may develop small to large lesions on their leaves. These lesions are typically brownish or grayish in colour which are irregular. Bandara et al. (2022) reported that a characteristic symptom of *Curvularia senegalensis* infection is the presence of white or grayish mold growth on the surface of leaves. Mattihalli et al. (2018) observed that for plants attacked by fungal pathogens still present few small spots on the leaves that enlarge over time and some spots coalesce or merge into blotches. Nalini and Parthasarathi (2018) reported that leaves and possibly the stems of infected plants lose turgidity, turn light green to greenish-yellow to brown, and finally collapse and die when they are attacked by *Fusarium*. According to Sharma et al. (2018) cabbage plants with *Fusarium* symptoms portray a lateral warping or curling of the stem and leaves occurs. The lower part of the leaf blade adjoining the petiole or midrib wilts and dies. The lower leaves turn yellow and later the upper leaves

are affected. With time, the yellow leaves turn brown and the affected tissue becomes dry and brittle (Jagadeesh et al., 2022). The speed of progress of disease in the plant depends upon the degree of varietal susceptibility and the soil temperature. According to Park et al. (2002). A Symptoms of *Aspergillus* on infected cabbage leaves is the development of small, circular, or irregularly shaped spots. These spots can be brown, dark brown, or black in color.

The results obtained on cultural characters matches with that of Hafizi et al. (2013) who recorded similar results when studying the morphological and molecular characterization of *Fusarium. solani* and *Fusarium.* oxysporum associated with crown disease of oil palm. Both cultural and morphological studies are essential for the identification of cabbage fungi pathogens. The variation in the different colony characteristics was an indication that cabbage in the field is affected by a diversity of fungi pathogens. The results were in line with those of Gherbawy et al. (2014). Also, the study was in line with that of Sharma et al. (2018), who reported that colonies appear initially white or off-white and gradually turns pinkish or purple over time. The texture of the colony may be fluffy or cottony. *Fusarium oxysporum* on PDA generally exhibits a moderate to fast growth rate, depending on the isolate.

The result based on molecular analysis identification technique showed the genera *Fusarium*, *Curvularia*, *Trichoderma*, *Alternaria*, *Aspergillus*, *Phoma* caused cabbage diseases. Part of this result is similar with that of Claassen, (2021), who reported that the black-leg disease of cabbage was caused by *Phoma lingam.* Also, the result is supported by Sultana (2022) who indicated that cabbage, quinoa and rice are affected by *Trichoderma harzianum*, *Aspergilus niger*, Fusarium sp., *Aspergilus flavus*, *Fusarium oxysporum.* The presence of *Trichoderma* sp. and *Fusarium* sp that was identified from diseased cabbage were supported with findings from the works of Adhikary et al. (2017), who concluded that *Fusarium* wilt in eggplants is caused by *Fusarium oxysporum* after conducting a pathogenicity test. The molecular results which identified *Trichoderma* sp. and *Penicillium* sp. to be responsible for cabbage diseases disagreed with that of Druzhinina et al. (2005), who reported that symptoms of Trichoderma, Hypocrea, or Trichophyton on cabbage are closely related to symptoms of species in the genera *Penicillium* (Geiser et al., 2007) and *Fusarium* (Chandra et al., 2011). The molecular results also tie with that of Blagojević et al. (2020) who reported that the *Alternaria* sp were responsible for the leaf spot disease of cabbage. The results did not match with that of Yao et al. (2023) who reported that *Trichoderma* species are use to control plant fungal and nematode diseases. The molecular identification method was supported by Sharma et al. (2013) who use PCR based assay for the detection of *Alternaria brassicicola* in crucifers in India. The results showed that *Fusarium oxysporum, Fusarium solani, Fusarium phillophilum, Fusarium nygam, and Fusarium graminearum* were monophyletic, suggesting that they share a common ancestral lineage.

Conclusion

The results from this study revealed that fungal diseases in the western highlands of Cameroon cause major loss in cabbage crop. Base on the cultural and morphological identification 81 isolated were identified to be attributed to fungi diseases of cabbage. Looking at the results of molecular analysis, 45 fungi isolates were identified belonging to four different orders namely, Hypocreales, Saccharomycetales, Eurotiales and Pleosporales. The most prevalent order was Hypocreales which was made up of *Trichoderma Sp* and *Fusarium* sp. However, some fungal pathogens represented themselves

only ones. This indicates that *Trichoderma Sp* and *Fusarium* sp were the major causes of fungal diseases on cabbage in the western highlands of Cameroon.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

NFS, TRK, MGA designed the study. NFS conducted the experiments and performed data analysis. NFS, TRK and MGA wrote the first draft. All authors contributed to and agreed on thefinal version.

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